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L6 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2000:528323 CAPLUS
DN 133:219839
TI **GPI** in lower animals
AU **Schofield, Louis**
CS The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, 3050, Australia
SO Paroxysmal Nocturnal Hemoglobinuria and the Glycosylphosphatidylinositol-Linked Proteins (2000), 179-198. Editor(s): Young, Neal S.; Moss, Joel. Publisher: Academic Press, San Diego, Calif.
CODEN: 69AEW3
DT Conference; General Review
LA English
AB A review with 113 refs. on glycosylphosphatidylinositol-linked proteins (**GPI**). (c) 2000 Academic Press.
RE.CNT 113 THERE ARE 113 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

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E3 41 --> SCHOFIELD LOUIS/AU
E4 175 SCHOFIELD M/AU
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DN 132:303491
TI A method of activating T cells with a glycosylphosphatidylinositol, and therapeutic use
IN **Schofield, Louis**; Hansen, Diana
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000024406	A1	20000504	WO 1999-AU929	19991027
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1126857	A1	20010829	EP 1999-970921	19991027
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRAI AU 1998-6758 A 19981027
WO 1999-AU929 W 19991027
AB The invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (**GPI**) mols. and derivs. or equiv. thereof. Even more particularly, the method of the invention contemplates a method of activating T cells, using **GPI** mols. via a CD1-restricted pathway. The method of the invention is useful for a range of therapeutic and/or prophylactic applications including e.g. applications which require skewing of the TH1/TH2 response or which require the induction of antibody prodn.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS
AN 2000:190951 CAPLUS
DN 132:235899
TI Immunogenic compositions and uses thereof
IN **Schofield, Louis**
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 101 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000015254	A1	20000323	WO 1999-AU770	19990914
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9958420	A1	20000403	AU 1999-58420	19990914

EP 1113815 A1 20010711 EP 1999-945777 19990914
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
PRAI AU 1998-5893 A 19980914
WO 1999-AU770 W 19990914
AB The present invention relates generally to a method of eliciting or otherwise inducing an effective immune response to a micro-organism and compns. for use therein. More particularly, the present invention relates to a method of inducing an immune response to a parasite utilizing an immunogenic compn. comprising a glycosylphosphatidylinositol (referred to herein as "**GPI**") inositolglycan domain or its derivs. Even more particularly, the present invention contemplates an immunogenic compn. comprising the Plasmodium falciparum **GPI** inositolglycan domain or its derivs. The present invention is useful, inter alia , as a prophylactic and/or therapeutic treatment for disease conditions such as, for example, infection by parasites and in particular infection by Plasmodium species.
RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 1993:141666 BIOSIS
DN PREV199395074466
TI Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites.
AU **Schofield, Louis (1)**; Hackett, Fiona
CS (1) Natl. Inst. Med. Res., The Ridgeway, Mill Hill, London NW7 1AA UK
SO Journal of Experimental Medicine, (1993) Vol. 177, No. 1, pp. 145-153. ISSN: 0022-1007.
DT Article
LA English
AB In this study, we have identified a dominant glycolipid toxin of Plasmodium falciparum. It is a glycosylphosphatidylinositol (**GPI**). The parasite **GPI** moiety, free or associated with protein, induces tumor necrosis factor and interleukin 1 production by macrophages and regulates glucose metabolism in adipocytes. Deacylation with specific phospholipases abolishes cytokine induction, as do inhibitors of protein kinase C. When administered to mice in vivo the parasite **GPI** induces cytokine release, a transient pyrexia, and hypoglycemia. When administered with sensitizing agents it can elicit a profound and lethal cachexia. Thus, the **GPI** of Plasmodium is a potent glycolipid toxin that may be responsible for a novel pathogenic process, exerting pleiotropic effects on a variety of host cells by substituting for the endogenous **GPI**-based second messenger/signal transduction pathways. Antibody to the **GPI** inhibits these toxic activities, suggesting a rational basis for the development of an antiglycolipid **vaccine** against malaria.

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FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 14:47:23 ON 06 MAY 2003
E SCHOFIELD LOUIS/AU
L1 9 S E1
L2 41 S E3
L3 26 S L2 AND GPI
L4 7 S L3 AND VACCIN?
L5 4 DUP REM L4 (3 DUPLICATES REMOVED)

=> dup rem l3

GPI vaccination could prevent pathology and fatalities in the Plasmodium berghei/rodent model of severe malaria. The P. falciparum **GPI** glycan of the sequence NH2-CH2-CH2-PO4-(Manalpha1-2)6Manalpha1-2Manalpha1-6Manalpha-1-4GlcNH2alpha1-6myo-inositol-1,2-cyclic-phosphate was chemically synthesized, conjugated to carriers, and used to immunize mice. Recipients were substantially protected against malarial acidosis, pulmonary oedema, cerebral syndrome and fatality. Anti-**GPI** antibodies neutralized pro-inflammatory activity by P. falciparum in vitro. Thus, we show that **GPI** is a significant pro-inflammatory endotoxin of parasitic origin, and that several disease parameters in malarious mice are toxin-dependent. **GPI** may contribute to pathogenesis and fatalities in humans. Synthetic **GPI** is therefore a prototype carbohydrate anti-toxic vaccine against malaria.

L6 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2000:290843 CAPLUS
DN 132:303491
TI A method of activating T cells with a glycosylphosphatidylinositol, and therapeutic use
IN **Schofield, Louis**; Hansen, Diana
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000024406	A1	20000504	WO 1999-AU929	19991027
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1126857	A1	20010829	EP 1999-970921	19991027
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI AU 1998-6758	A	19981027		
WO 1999-AU929	W	19991027		

AB The invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (**GPI**) mols. and derivs. or equiv. thereof. Even more particularly, the method of the invention contemplates a method of activating T cells, using **GPI** mols. via a CD1-restricted pathway. The method of the invention is useful for a range of therapeutic and/or prophylactic applications including e.g. applications which require skewing of the TH1/TH2 response or which require the induction of antibody prodn.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2000:190951 CAPLUS
DN 132:235899
TI Immunogenic compositions and uses thereof
IN **Schofield, Louis**
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 101 pp.
CODEN: PIXXD2

AB In this study we demonstrate that glycosylphosphatidylinositol (**GPI**) of malaria parasite origin directly increases cell adhesion molecule expression in purified HUVECs in a dose- and time-dependent manner, resulting in a marked increase in parasite and leukocyte cytoadherence to these target cells. The structurally related glycolipids dipalmitoyl-phosphatidylinositol and iM4 glycoinositolphospholipid of Leishmania mexicana had no such activity. Malarial **GPI** exerts this effect by activation of an endogenous **GPI**-based signal transduction pathway in endothelial cells. **GPI** induces rapid onset tyrosine phosphorylation of multiple intracellular substrates within 1 min of addition to cells in a dose-dependent manner. This activity can be blocked by the protein tyrosine kinase-specific antagonist herbimycin A, genistein, and tyrphostin. These tyrosine kinase antagonists also inhibit **GPI**-mediated up-regulation of adhesin expression and parasite cytoadherence. **GPI**-induced up-regulation of adhesin expression and parasite cytoadherence can also be blocked by the NF-kappa-B/c-rel antagonist pyrrolidine-dithiocarbamate, suggesting the involvement of this family of transcription factors in **GPI** -induced adhesin expression. The direct activation of endothelial cells by **GPI** does not require the participation of TNF or IL-1. However, **GPI** is also responsible for the indirect pathway of increased adhesin expression mediated by TNF and IL-1 output from monocytes/macrophages. Total parasite extracts also up-regulate adhesin expression and parasite cytoadherence in HUVECs, and this activity is blocked by a neutralizing mAb to malarial **GPI**, suggesting that **GPI** is the dominant agent of parasite origin responsible for this activity. Thus, a parasite-derived **GPI** toxin activates vascular endothelial cells by tyrosine kinase-mediated signal transduction, leading to NF-kappa-B/c-rel activation and downstream expression of adhesins, events that may play a central role in the etiology of cerebral malaria.

L6 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8

AN 1996:244187 BIOSIS

DN PREV199698792316

TI Structural analysis of the glycosyl-phosphatidylinositol membrane anchor of the merozoite surface proteins-1 and -2 of Plasmodium falciparum.

AU Gerold, Peter; **Schofield, Louis**; Blackman, Michael J.; Holder, Anthony A.; Schwarz, Ralph T.

CS Zentrum fuer Hygiene und Med. Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch Str. 17 Zentrum fuer Hygiene und Med. Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch Str. 17 Germany

SO Molecular and Biochemical Parasitology, (1996) Vol. 75, No. 2, pp. 131-143.
ISSN: 0166-6851.

DT Article

LA English

AB Plasmodium falciparum accumulates the two merozoite surface proteins-1 and -2 during schizogony. Both proteins are proposed to be anchored in membranes by glycosyl-phosphatidylinositol membrane anchors. In this report the identity of these **GPI**-anchors is confirmed by labelling with tritiated precursors and additionally by specific enzymatic and chemical treatments. Detailed structural analysis of the core-glycans showed that the **GPI**-anchors of both proteins possess an extra alpha-1-2 linked mannose at the conserved trimannosyl-core-glycan. MSP-1 and MSP-2 labelled with tritiated myristic acid possess primarily radioactive myristic acid at inositol rings in both **GPI**-anchors. Additionally the hydrophobic fragments released from (3H)myristic acid labelled **GPI**-anchors were identified as diacyl-glycerols, carrying preferentially (3H)palmitic acid in an ester-linkage.

L6 ANSWER 13 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9

AN 1995:79000 BIOSIS
DN PREV199598093300
TI Glycosylphosphatidylinositol toxin of Trypanosoma brucei regulates IL-1-alpha and TNF-alpha expression in macrophages by protein tyrosine kinase mediated signal transduction.
AU Tachado, Souvenir D. (1); **Schofield, Louis**
CS (1) Walter and Eliza Hall Inst. Med. Res., Post Office, Royal Melbourne Hosp., Parkville 3050, Victoria Australia
SO Biochemical and Biophysical Research Communications, (1994) Vol. 205, No. 2, pp. 984-991.
ISSN: 0006-291X.
DT Article
LA English
AB A purified, structurally defined glycosylphosphatidylinositol (**GPI**) derived from the Variant Surface Glycoprotein (VSG) of Trypanosoma brucei, and its biosynthetic precursor P2, was able at submicromolar concentrations to regulate cytokine expression when added directly as pharmacological agonist to host macrophages, by activation of an endogenous protein tyrosine-kinase (PTK) mediated signal transduction pathway. **GPI** induces rapid onset tyrosine phosphorylation of multiple intracellular substrates, within minutes of addition to LPS-nonresponsive cells, followed shortly thereafter by IL-1-alpha secretion. The PTK antagonists genistein and tyrphostin inhibit both tyrosylphosphorylation and cytokine expression. A monoclonal antibody to **GPI** also blocks IL-1-alpha induction by total parasite extracts. Thus, as in malaria infection, **GPI** may induce the cytokine excess causing certain pathological states associated with trypanosomiasis.

L6 ANSWER 14 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 1993:141666 BIOSIS
DN PREV199395074466
TI Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites.
AU **Schofield, Louis (1)**; Hackett, Fiona
CS (1) Natl. Inst. Med. Res., The Ridgeway, Mill Hill, London NW7 1AA UK
SO Journal of Experimental Medicine, (1993) Vol. 177, No. 1, pp. 145-153.
ISSN: 0022-1007.
DT Article
LA English
AB In this study, we have identified a dominant glycolipid toxin of Plasmodium falciparum. It is a glycosylphosphatidylinositol (**GPI**). The parasite **GPI** moiety, free or associated with protein, induces tumor necrosis factor and interleukin 1 production by macrophages and regulates glucose metabolism in adipocytes. Deacylation with specific phospholipases abolishes cytokine induction, as do inhibitors of protein kinase C. When administered to mice in vivo the parasite **GPI** induces cytokine release, a transient pyrexia, and hypoglycemia. When administered with sensitizing agents it can elicit a profound and lethal cachexia. Thus, the **GPI** of Plasmodium is a potent glycolipid toxin that may be responsible for a novel pathogenic process, exerting pleiotropic effects on a variety of host cells by substituting for the endogenous **GPI**-based second messenger/signal transduction pathways. Antibody to the **GPI** inhibits these toxic activities, suggesting a rational basis for the development of an antiglycolipid vaccine against malaria.

=> s gpi or inositolglycan domain or inositolglycan
L7 19791 GPI OR INOSITOLGLYCAN DOMAIN OR INOSITOLGLYCAN

=> s l7 and (parasit? or plasmodium)_

SL English; Portuguese
AB To understand the interaction of **Trypanosoma** cruzi and the immune system of the vertebrate host, and therefore the pathophysiology of Chagas' disease, different research groups have focused their attention on the identification and characterization of **parasite** molecules involved in the activation of either innate or adaptive immune responses. The **parasite** surface molecules that serve as targets of the vertebrate host immune system have also been studied and identified. These studies have revealed that the quantitatively dominant complex of glycosylphosphatidylinositol (**GPI**)-anchored molecules (GIPLs, mucins and TS) present on the surface of T. cruzi trypomastigotes are essential to control activation of the innate immune system and promote initiation of acquired immune responses in the vertebrate host. Two major families of surface glycoproteins (mucin-like glycoproteins and transialidases) have also been shown to be important targets of **parasite** specific humoral and cellular immune responses. They are, thus, important candidates for **vaccine** development as determined in studies using experimental models. Studies regarding the molecular cloning and/or biochemical characterization of the above mentioned T. cruzi surface molecules, and their ability to influence the outcome of T. cruzi infection in the vertebrate host through the stimulation and/or control of the immune system are presently reviewed. A proposition is made that such molecules may have evolved and been selectively conserved to establish an equilibrium between the **parasite** and its vertebrate host, limiting **parasite** replication, but allowing **parasite** persistence and host survival, thus favoring the maintenance of T. cruzi life cycle.

L11 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:227909 BIOSIS
DN PREV200000227909
TI Processing and localisation of a **GPI**-anchored **Plasmodium** falciparum surface protein expressed by the baculovirus system.
AU Kedees, Mamdouh H.; Gerold, Peter; Azzouz, Nahid; Blaschke, Thomas; Shams-Eldin, Hosam; Muehlberger, Elke; Holder, Anthony A.; Klenk, Hans-Dieter; Schwarz, Ralph T. (1); Eckert, Volker
CS (1) Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg Germany
SO European Journal of Cell Biology, (Jan, 2000) Vol. 79, No. 1, pp. 52-61. ISSN: 0171-9335.
DT Article
LA English
SL English
AB We describe the expression, in insect cells using the baculovirus system, of two protein fragments derived from the C-terminus of merozoite surface protein 1 (MSP-1) of the human malaria **parasite** **Plasmodium** falciparum, and their glycosylation and intracellular location. The transport and intracellular localisation of the intact C-terminal MSP-1 fragment, modified by addition of a signal sequence for secretion, was compared with that of a similar control protein in which translation of the **GPI**-cleavage/attachment site was abolished by insertion of a stop codon into the DNA sequence. Both proteins could only be detected intracellularly, most likely in the endoplasmic reticulum.. This lack of transport to the cell surface or beyond, was confirmed for both proteins by immunofluorescence with a specific antibody and characterisation of their N-glycans. The N-glycans had not been processed by enzymes localised in post-endoplasmic reticulum compartments. In contrast to MSP-1, the surface antigen SAG-1 of **Toxoplasma** gondii was efficiently transported out of the endoplasmic reticulum of insect cells and was located, at least in part, on the cell surface. No **GPI**-anchor could be detected for either of the MSP-1 constructs or SAG-1, showing that the difference in transport is a property of the

serum cleaved phosphatidic acid from the 200 000 MW protein. Although the functional significance of these **GPI**-anchored proteins is unknown, it is suggested that their release from the surface of the schistosome may contribute to immune evasion.

L11 ANSWER 8 OF 12 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-271259 [23] WPIDS
DNC C2000-082769
TI Inducing immune response against a microorganism, useful particularly for treating or preventing malaria, by administering an **inositolglycan domain** of glycosylphosphatidylinositol.
DC B04 D16
IN SCHOFIELD, L
PA (HALL-N) HALL INST MEDICAL RES WALTER & ELIZA
CYC 90
PI WO 2000015254 A1 20000323 (200023)* EN 100p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9958420 A 20000403 (200034)
EP 1113815 A1 20010711 (200140) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT WO 2000015254 A1 WO 1999-AU770 19990914; AU 9958420 A AU 1999-58420 19990914; EP 1113815 A1 EP 1999-945777 19990914, WO 1999-AU770 19990914
FDT AU 9958420 A Based on WO 200015254; EP 1113815 A1 Based on WO 200015254
PRAI AU 1998-5893 19980914
AB WO 200015254 A UPAB: 20000516
NOVELTY - Method of eliciting or inducing, in a mammal, an immune response against a microorganism comprises administering a composition (A), containing a compound (I) that induces a response to the **inositolglycan domain** of a **GPI** (glycosylphosphatidylinositol), but not to the lipid domain of **GPI**

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of therapeutically or prophylactically treating microorganism infection by administering (A);

(2) the composition (A);

(3) a **vaccine** or pharmaceutical composition containing (I) and at least one carrier and/or diluent;

(4) an antibody (Ab) directed to the **inositolglycan domain** of **GPI**, which does not interact with the lipid domain;

(5) a pharmaceutical composition containing Ab and at least one carrier and/or diluent; and

(6) a method of inhibiting, halting or delaying mammalian disease onset or progression, caused by microorganism infection, by administering Ab.

ACTIVITY - Antimicrobial; antifungal; protozoacide. Mice were infected with 1 million **Plasmodium** bergheri ANKA, then after 4 days treated daily (for 7 days) with 0.1 mu g/day (intraperitoneally) of the monoclonal antibody 1D12 (raised in mice against a conjugate of **Plasmodium GPI** glycan domain and ovalbumin). All untreated controls died of cerebral malaria 6-8 days after infection, but all treated animals were still alive after 14 days.

MECHANISM OF ACTION - **Vaccine**. **GPI** is a toxin that regulates host cell function and gene expression, resulting in activation of transcription factors that upregulate expression of proinflammatory molecules. (I) reduce both the toxic and immunosuppressive

effects of **GPI** or it kills microbial **parasites** directly.

USE - (A) is used to treat and/or prevent microbial infections, especially **Plasmodium** infections, and malaria (claimed), but also infection by **Trypanosoma**, Leishmania, **Toxoplasma** and **Candida**. Antibodies (Ab) directed against the **inositolglycan domain** can also be used to treat or prevent infections, and as immunoassay reagents for detecting the **GPI** domain, e.g. for diagnosis of **parasitic** infection, autoimmune and degenerative diseases, and somatic acquired genetic defects, or for monitoring therapy, and for affinity purification of recombinant **GPI**.

ADVANTAGE - Fragments of **GPI** that lack the lipid domain induce a protective response, although those that contain the lipid domain do not, and produce an immunoglobulin M that exacerbates the disease.

Dwg.0/12

L11 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 2000:290843 CAPLUS
DN 132:303491
TI A method of activating T cells with a glycosylphosphatidylinositol, and therapeutic use
IN Schofield, Louis; Hansen, Diana
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000024406	A1	20000504	WO 1999-AU929	19991027
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1126857	A1	20010829	EP 1999-970921	19991027
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI AU 1998-6758	A	19981027		
WO 1999-AU929	W	19991027		

AB The invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (**GPI**) mols. and derivs. or equiv. thereof. Even more particularly, the method of the invention contemplates a method of activating T cells, using **GPI** mols. via a CD1-restricted pathway. The method of the invention is useful for a range of therapeutic and/or prophylactic applications including e.g. applications which require skewing of the TH1/TH2 response or which require the induction of antibody prodn.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 1995:319826 CAPLUS
DN 122:98808
TI Cloning and expression of human .beta.2-microglobulin cDNA and the construction of fusion proteins between antigenic epitopes and

.beta.2-microglobulin
IN Edwards, Richard Mark; Hunter, Michael George
PA British Bio-Technology Ltd., UK
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9424290	A1	19941027	WO 1994-GB755	19940408
	W: AU, BR, CA, CN, CZ, DE, FI, GB, HU, JP, KR, NO, NZ, PL, RU, UA, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9464353	A1	19941108	AU 1994-64353	19940408
	EP 693125	A1	19960124	EP 1994-912040	19940408
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
	US 2002123108	A1	20020905	US 1995-532549	19951201
PRAI	GB 1993-7371	A	19930408		
	WO 1994-GB755	W	19940408		

AB A method is described for the cloning and expression of human .beta.2-microglobulin (B2M) cDNA in vector host cells which allows the construction of B2M fusion proteins with antigenic sequences from various etiol. agents or tumors. Preferred antigenic sequences are derived from the third variable domain (V3 loop) of an envelope protein of a lentivirus. These fusion proteins can be used as prophylactic or immunotherapeutic **vaccines** to induce neutralizing antibody responses. Thus, B2M cDNA was inserted into the pHILD1 expression vector for expression in the Pichia pastoris system. The expression vector includes an AOX promoter sequence and an .alpha.-factor or Pho1 leader sequence to obtain secretion of the fusion protein from the yeast cells. Within the Pichia pastoris expression system, the B2M gene was fused at its 5' end to the Sendai virus epitope (FAPGNYPAL-GGGGG, where the pentaglycine is a short linker) or to the influenza A virus nucleoprotein epitope (GILGFVFTL-GGGGGGSSS). Prodn. levels from strains with the .alpha.-factor leader sequence were .apprx.150 mg/L. The hybrid Sendai-B2M product was shown to induce Sendai nucleoprotein-specific cytotoxic T-lymphocytes.

L11 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS
AN 1995:154369 CAPLUS
DN 122:79112
TI Self-assembling protein particles presenting foreign epitopes on their surfaces
IN Adams, Sally Elizabeth; Burns, Robert Nigel; Richardson, Simon Mark Harold
PA British Bio-Technology Ltd., UK
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9414969	A1	19940707	WO 1993-GB2656	19931224
	W: AU, CA, CZ, DE, FI, GB, HU, JP, KR, NO, NZ, RU, UA, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9457112	A1	19940719	AU 1994-57112	19931224
	EP 677111	A1	19951018	EP 1994-902961	19931224
	EP 677111	B1	19970514		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
	JP 08505768	T2	19960625	JP 1993-514974	19931224
	AT 153071	E	19970515	AT 1994-902961	19931224
	ES 2104339	T3	19971001	ES 1994-902961	19931224

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     US 6060064        A    20000509       US 1995-492076   19950628
PRAI GB 1992-27068     A    19921229
     WO 1993-GB2656    W    19931224
AB   Fusion proteins of the yeast retrotransposon Ty p1 protein and an
     antigenic peptide that self-assemble into particles presenting the epitope
     on the outside are prepd. for use in vaccines. This is achieved
     by substituting the exposed immunodominant epitope of Ty p1 with the
     foreign peptide. The epitopes of the p1 protein were identified by std.
     methods of epitope scanning and three peptides were identified and those
     in the N-terminal region were found to be exposed on the surface of the
     protein. The TyA gene encoding the protein was manipulated to introduce
     convenient NheI sites in the epitope coding regions and the protein
     encoded by expression of these genes continued to self-assoc. A series of
     analogs with peptides from the V3 loop of gp120 of HIV-1 inserted at the
     epitope sites were prepd. by expression of the gene in yeast and tested
     for their response to anti-gp120 antisera. These proteins self-assembled.
     The fusion proteins reacted with antisera to the p1 protein and to the V3
     epitope; different antisera responded to the V3 epitope in different
     positions.

L11  ANSWER 12 OF 12  CAPLUS  COPYRIGHT 2003 ACS
AN   1994:673849  CAPLUS
DN   121:273849
TI   Manufacture of antigens in gag protein-based particles using a minimal
     retroviral expression cassette
IN   Czaplewski, Lloyd George
PA   British Bio-Technology Ltd., UK
SO   PCT Int. Appl., 40 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 9420621         A2   19940915       WO 1994-GB281    19940211
     WO 9420621         A3   19941013
         W:  AU, CA, CN, DE, FI, GB, JP, KR, NO, NZ, RU, UA, US
         RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
     AU 9460063         A1   19940926       AU 1994-60063    19940211
PRAI GB 1993-4239            19930301
     WO 1994-GB281           19940211
AB   An expression cassette using a single promoter to drive expression of a
     gag-derived sequence from a complex retrovirus including a rev gene, an
     RRE element and donor and acceptor elements is described for use in the
     manuf. of retroviral particles presenting antigens for use in
     vaccines. The construct is arranged to ensure that the promoter
     is capable of driving expression of both the gag-derived sequence and the
     rev-like element. The construct does not contain a functional env gene.
     The construction of a series of such cassettes for the synthesis of tat
     protein is demonstrated. COS-7 cells co-transfected with one of these
     constructs and a CAT gene under control of a tat-responsive promoter
     showed high levels of expression of the CAT gene.


=> d his

     (FILE 'HOME' ENTERED AT 14:46:32 ON 06 MAY 2003)

     FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,
     LIFESCI, CAPLUS' ENTERED AT 14:47:23 ON 06 MAY 2003
                E SCHOFIELD LOUIS/AU
L1              9 S E1
L2             41 S E3
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L3            26 S L2 AND GPI
L4             7 S L3 AND VACCIN?
L5             4 DUP REM L4 (3 DUPLICATES REMOVED)
L6            14 DUP REM L3 (12 DUPLICATES REMOVED)
L7         19791 S GPI OR INOSITOLGLYCAN DOMAIN OR INOSITOLGLYCAN
L8          1890 S L7 AND (PARASIT? OR PLASMODIUM)
L9           767 S L8 AND (TRYPANOSOMA OR LEISHMAINA OR TOXOPLASMA OR CANDIDA O
L10          281 DUP REM L9 (486 DUPLICATES REMOVED)
L11           12 S L10 AND VACCIN?

=> s l9 and malaria
L12           45 L9 AND MALARIA

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           14 DUP REM L12 (31 DUPLICATES REMOVED)

=> d bib ab 1-14

L13  ANSWER 1 OF 14  WPIDS (C) 2003 THOMSON DERWENT
AN   2000-271259 [23]   WPIDS
DNC  C2000-082769
TI   Inducing immune response against a microorganism, useful particularly for
     treating or preventing malaria, by administering an
     inositolglycan domain of glycosylphosphatidylinositol.
DC   B04 D16
IN   SCHOFIELD, L
PA   (HALL-N) HALL INST MEDICAL RES WALTER & ELIZA
CYC  90
PI   WO 2000015254 A1 20000323 (200023)* EN  100p.
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
            OA PT SD SE SL SZ TZ UG ZW
         W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
            FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
            LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ
            TM TR TT TZ UA UG US UZ VN YU ZA ZW
     AU 9958420    A  20000403 (200034)
     EP 1113815    A1 20010711 (200140)  EN
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI
ADT  WO 2000015254 A1 WO 1999-AU770 19990914; AU 9958420 A AU 1999-58420
     19990914; EP 1113815 A1 EP 1999-945777 19990914, WO 1999-AU770 19990914
FDT  AU 9958420 A Based on WO 200015254; EP 1113815 A1 Based on WO 200015254
PRAI AU 1998-5893     19980914
AB   WO 200015254 A UPAB: 20000516
     NOVELTY - Method of eliciting or inducing, in a mammal, an immune response
     against a microorganism comprises administering a composition (A),
     containing a compound (I) that induces a response to the
     inositolglycan domain of a GPI
     (glycosylphosphatidylinositol), but not to the lipid domain of GPI

          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
     following:
          (1) a method of therapeutically or prophylactically treating
     microorganism infection by administering (A);
          (2) the composition (A);
          (3) a vaccine or pharmaceutical composition containing (I) and at
     least one carrier and/or diluent;
          (4) an antibody (Ab) directed to the inositolglycan
     domain of GPI, which does not interact with the lipid
     domain;
          (5) a pharmaceutical composition containing Ab and at least one
     carrier and/or diluent; and
```

(6) a method of inhibiting, halting or delaying mammalian disease onset or progression, caused by microorganism infection, by administering Ab.

ACTIVITY - Antimicrobial; antifungal; protozoacide. Mice were infected with 1 million **Plasmodium** bergheri ANKA, then after 4 days treated daily (for 7 days) with 0.1 mu g/day (intraperitoneally) of the monoclonal antibody 1D12 (raised in mice against a conjugate of **Plasmodium GPI** glycan domain and ovalbumin). All untreated controls died of cerebral **malaria** 6-8 days after infection, but all treated animals were still alive after 14 days.

MECHANISM OF ACTION - Vaccine. **GPI** is a toxin that regulates host cell function and gene expression, resulting in activation of transcription factors that upregulate expression of proinflammatory molecules. (I) reduce both the toxic and immunosuppressive effects of **GPI** or it kills microbial **parasites** directly.

USE - (A) is used to treat and/or prevent microbial infections, especially **Plasmodium** infections, and **malaria** (claimed), but also infection by **Trypanosoma**, Leishmania, **Toxoplasma** and **Candida**. Antibodies (Ab) directed against the **inositolglycan domain** can also be used to treat or prevent infections, and as immunoassay reagents for detecting the **GPI** domain, e.g. for diagnosis of **parasitic** infection, autoimmune and degenerative diseases, and somatic acquired genetic defects, or for monitoring therapy, and for affinity purification of recombinant **GPI**.

ADVANTAGE - Fragments of **GPI** that lack the lipid domain induce a protective response, although those that contain the lipid domain do not, and produce an immunoglobulin M that exacerbates the disease.

Dwg.0/12

L13 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 2000:290843 CAPLUS
DN 132:303491
TI A method of activating T cells with a glycosylphosphatidylinositol, and therapeutic use
IN Schofield, Louis; Hansen, Diana
PA The Walter and Eliza Hall Institute of Medical Research, Australia
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000024406	A1	20000504	WO 1999-AU929	19991027
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1126857	A1	20010829	EP 1999-970921	19991027
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI	AU 1998-6758	A	19981027		
	WO 1999-AU929	W	19991027		

AB The invention relates generally to a method of activating T cells and more particularly to a method of activating T cells using glycosylphosphatidylinositol (**GPI**) mols. and derivs. or equiv. thereof. Even more particularly, the method of the invention contemplates

a method of activating T cells, using **GPI** mols. via a CD1-restricted pathway. The method of the invention is useful for a range of therapeutic and/or prophylactic applications including e.g. applications which require skewing of the TH1/TH2 response or which require the induction of antibody prodn.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

AN 2000:227909 BIOSIS

DN PREV200000227909

TI Processing and localisation of a **GPI**-anchored **Plasmodium** falciparum surface protein expressed by the baculovirus system.

AU Kedees, Mamdouh H.; Gerold, Peter; Azzouz, Nahid; Blaschke, Thomas; Shams-Eldin, Hosam; Muehlberger, Elke; Holder, Anthony A.; Klenk, Hans-Dieter; Schwarz, Ralph T. (1); Eckert, Volker

CS (1) Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg Germany

SO European Journal of Cell Biology, (Jan, 2000) Vol. 79, No. 1, pp. 52-61. ISSN: 0171-9335.

DT Article

LA English

SL English

AB We describe the expression, in insect cells using the baculovirus system, of two protein fragments derived from the C-terminus of merozoite surface protein 1 (MSP-1) of the human **malaria parasite** **Plasmodium** falciparum, and their glycosylation and intracellular location. The transport and intracellular localisation of the intact C-terminal MSP-1 fragment, modified by addition of a signal sequence for secretion, was compared with that of a similar control protein in which translation of the **GPI**-cleavage/attachment site was abolished by insertion of a stop codon into the DNA sequence. Both proteins could only be detected intracellularly, most likely in the endoplasmic reticulum. This lack of transport to the cell surface or beyond, was confirmed for both proteins by immunofluorescence with a specific antibody and characterisation of their N-glycans. The N-glycans had not been processed by enzymes localised in post-endoplasmic reticulum compartments. In contrast to MSP-1, the surface antigen SAG-1 of **Toxoplasma** gondii was efficiently transported out of the endoplasmic reticulum of insect cells and was located, at least in part, on the cell surface. No **GPI**-anchor could be detected for either of the MSP-1 constructs or SAG-1, showing that the difference in transport is a property of the individual proteins and cannot be attributed to the lack of a **GPI** -anchor. The different intracellular location and post-translational modification of recombinant proteins expressed in insect cells, as compared to the native proteins expressed in **parasites**, and the possible implications for vaccine development are discussed.

L13 ANSWER 4 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

AN 2000:88912 BIOSIS

DN PREV200000088912

TI Specificity in signal transduction among glycosylphosphatidylinositols of **Plasmodium** falciparum, **Trypanosoma** brucei, **Trypanosoma** cruzi and Leishmania spp.

AU Tachado, Souvenir D.; Mazhari-Tabrizi, Ramin; Schofield, Louis (1)

CS (1) Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville, VIC, 3050 Australia

SO Parasite Immunology (Oxford), (Dec., 1999) Vol. 21, No. 12, pp. 609-617. ISSN: 0141-9838.

DT Article

LA English
SL English
AB Glycosylphosphatidylinositols (GPIs) and related glycoconjugates of **parasite** origin have been shown to regulate both the innate and acquired immune systems of the host. This is achieved through the activation of novel **GPI**-dependent signalling pathways in macrophages, lymphocytes and other cell types. **Parasite** GPIs impart at least two distinct signals to host cells through the structurally distinct inositolphosphoglycan (IPG) and fatty acid domains. Binding of IPG to as yet uncharacterized cell surface receptor(s) leads to activation of src-family protein tyrosine kinases: depending upon structure, **GPI**-derived fatty acids can either activate or antagonize protein kinase C, and may enter the sphingomyelinase pathway. The degree of fatty acid saturation may also contribute to signalling activity. Thus, variation in structure of **parasite** GPIs imparts different properties of signal transduction upon this class of glycolipid. The divergent activities of GPIs from various protozoal taxa reflect global aspects of the host/**parasite** relationship, suggesting that **GPI** signalling is a central determinant of disease in **malaria**, leishmaniasis and both American and African trypanosomiases.

L13 ANSWER 5 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1999:194036 BIOSIS
DN PREV199900194036
TI The biosynthesis and post-translational modification of Pbs21 an ookinete-surface protein of **Plasmodium** berghei.
AU Alejo Blanco, A. Richard; Paez, Andres; Gerold, Peter; Dearsly, A. Louise; Margos, Gabriele; Schwarz, Ralph T.; Barker, Guy; Rodriguez, Maria C.; Sinden, Robert E. (1)
CS (1) Infection and Immunity Section, Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London, SW7 2BB UK
SO Molecular and Biochemical Parasitology, (Jan. 25, 1999) Vol. 98, No. 2, pp. 163-173.
ISSN: 0166-6851.
DT Article
LA English
AB Radiolabelled methionine incorporation into synchronised **Plasmodium** berghei gametocytes or ookinete cultures, showed that Pbs21 is not synthesised in bloodstage **parasites**; synthesis was detected within three hours of induction of gametogenesis; synthesis was triggered at gametogenesis, not by fertilisation. We show native Pbs21 to be a hydrophobic membrane protein that was insensitive to cleavage by phosphatidylinositol phospholipase C (PI-PLC), but sensitive to alkaline hydroxylamine, and partially sensitive to glycosylphosphatidylinositol-dependent phospholipase D (**GPI**-PLD) and HNO2. 3H-myristic and palmitic acid, 3H-glucosamine and mannose incorporation indicated Pbs21 was acylated and glycosylated. Linkage of the acyl group was sensitive to HNO2, which released an acyl-phosphatidylinositol more hydrophobic than that released from P3 of **Trypanosoma** brucei. All these properties are consistent with the presence of a **malaria** -specific glycosylphosphatidylinositol (**GPI**) anchor. In contrast recombinant Pbs21 (rPbs21), expressed in Spodoptera frugiperda cells, was sensitive to both PI-PLC and **GPI**-PLD, consistent with the protein being modified by a different (S. frugiperda) **GPI** anchor. Brefeldin A blocked secretion of rPbs21 within a cytoplasmic reticular compartment. Following deletion of the putative **GPI** anchor addition site (amino acids 189-213), the protein was transported to the cell surface and secreted directly into the aqueous phase of the culture medium. Deletion of amino acids 205-213 disrupted Pbs21 processing, transport through the ER and distribution onto the cell

surface. Deletion of amino acids 1-28 prevented transport of Pbs21 into the ER. This suggests that correct processing of the **GPI** anchor in the ER-Golgi network is essential for the successful secretion of the recombinant protein, which is additionally dependent upon an N-terminal secretory signal sequence.

L13 ANSWER 6 OF 14 WPIDS (C) 2003 THOMSON DERWENT DUPLICATE 4
AN 1999-070151 [06] WPIDS
DNN N1999-051369 DNC C1999-020685
TI New human glycosyl-phosphatidylinositol (GPI2) homologue (**GPI**-2h) - useful in the diagnosis, prevention and treatment of disorders related to **GPI**-anchored proteins, and **fungal**/**parasitic** infections.
DC B04 C06 D16 S03
IN BANDMAN, O; CORLEY, N C; SHAH, P
PA (INCY-N) INCYTE PHARM INC
CYC 42
PI WO 9853070 A1 19981126 (199906)* EN 63p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AT AU BR CA CH CN DE DK ES FI GB IL JP KR MX NO NZ RU SE SG US
AU 9875923 A 19981211 (199917)
US 5968742 A 19991019 (199950)
ADT WO 9853070 A1 WO 1998-US10527 19980522; AU 9875923 A AU 1998-75923 19980522; US 5968742 A US 1997-861512 19970522
FDT AU 9875923 A Based on WO 9853070
PRAI US 1997-861512 19970522
AB WO 9853070 A UPAB: 19990316
A human GPI2 homologue (I) (designated **GPI**-2h), including fragments, is new. Also claimed are: (1) a polynucleotide sequence (II), including variants, that encodes homologue (I), and acts as a hybridization probe; (2) a polynucleotide sequence, which is complementary to sequence (II), and hybridizes under stringent conditions; (3) an expression vector containing sequence (II); (4) a host cell containing the vector; (5) an antibody that binds to homologue (I); (6) an antagonist that binds to and controls the activity of homologue (I); and (7) identification of antifungal/antiprotozoal agents using homologue (I) and a **fungal**/protozoal GPI2.

USE - **GPI**-2h can be used as a pharmaceutical composition in the diagnosis, prevention and treatment of disorders related to **GPI**-anchored proteins, in addition to **fungal**/**parasitic** infections. These include paroxysmal nocturnal haemoglobinuria (PNH), diseases caused by protozoal **parasites** (**malaria**, African sleeping sickness, cattle disease nagama, Chagas' disease, kala azar, espundia and Oriental sore), and **fungal** infections, especially in individuals undergoing immunosuppressive therapy (Histoplasma sp., Coccidioides immitis, **Candida** and Aspergillus sp.).

ADVANTAGE - The production of **GPI** is essential to yeast and **parasitic** viability and infectivity. Therefore, **GPI**-2h provides a way of diagnosing, treating and preventing diseases related to this protein in mammals, fungi and protozoal **parasites**.
Dwg.0/10

L13 ANSWER 7 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 1997:212809 BIOSIS
DN PREV199799519313
TI Signal transduction in macrophages by glycosylphosphatidylinositols of **Plasmodium**, **Trypanosoma**, and Leishmania: Activation of protein tyrosine kinases and protein kinase C by **inositolglycan** and diacylglycerol moieties.
AU Tachado, Souvenir D. (1); Gerold, Peter; Schwarz, Ralph; Novakovic,

DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9424290	A1	19941027	WO 1994-GB755	19940408
	W: AU, BR, CA, CN, CZ, DE, FI, GB, HU, JP, KR, NO, NZ, PL, RU, UA, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9464353	A1	19941108	AU 1994-64353	19940408
	EP 693125	A1	19960124	EP 1994-912040	19940408
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
	US 2002123108	A1	20020905	US 1995-532549	19951201
PRAI	GB 1993-7371	A	19930408		
	WO 1994-GB755	W	19940408		

AB A method is described for the cloning and expression of human .beta.2-microglobulin (B2M) cDNA in vector host cells which allows the construction of B2M fusion proteins with antigenic sequences from various etiol. agents or tumors. Preferred antigenic sequences are derived from the third variable domain (V3 loop) of an envelope protein of a lentivirus. These fusion proteins can be used as prophylactic or immunotherapeutic vaccines to induce neutralizing antibody responses. Thus, B2M cDNA was inserted into the pHILD1 expression vector for expression in the Pichia pastoris system. The expression vector includes an AOX promoter sequence and an .alpha.-factor or Pho1 leader sequence to obtain secretion of the fusion protein from the yeast cells. Within the Pichia pastoris expression system, the B2M gene was fused at its 5' end to the Sendai virus epitope (FAPGNYPAL-GGGGG, where the pentaglycine is a short linker) or to the influenza A virus nucleoprotein epitope (GILGFVFTL-GGGGGGSSS). Prodn. levels from strains with the .alpha.-factor leader sequence were .apprx.150 mg/L. The hybrid Sendai-B2M product was shown to induce Sendai nucleoprotein-specific cytotoxic T-lymphocytes.

L13 ANSWER 10 OF 14 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 94319652 EMBASE
DN 1994319652
TI Toxicity of myristic acid analogs toward African trypanosomes.
AU Doering T.L.; Lu T.; Werbovetz K.A.; Gokel G.W.; Hart G.W.; Gordon J.I.; Englund P.T.
CS Department of Biological Chemistry, Johns Hopkins School of Medicine,Baltimore, MD 21205, United States
SO Proceedings of the National Academy of Sciences of the United States of America, (1994) 91/21 (9735-9739).
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal; Article
FS 004 Microbiology
037 Drug Literature Index
LA English
SL English
AB New drugs are needed for treatment of diseases caused by African trypanosomes. One possible target for chemotherapy is the biosynthesis of the glycosyl phosphatidylinositol (**GPI**) of this **parasite** 's variant surface glycoprotein (VSG). Unlike mammalian GPIs, the diacylglycerol moiety of the VSG anchor contains only myristate (tetradecanoate), added in unique remodeling reactions. We previously found that 11-oxatetradecanoic acid [i.e., 10-(propoxy)decanoic acid] is selectively toxic to trypanosomes. We have now assayed 244 different fatty acid analogs, most with chain lengths comparable to that of myristate, for trypanocidal effects. In these assays we surveyed the effects on toxicity of systematic alterations in the analogs' steric, conformational, and hydrophobic properties. We also used three 3H- labeled oxatetradecanoic acids to explore the mechanism of analog action. Their incorporation into VSG correlated roughly with toxicity, although they also were incorporated

that of the two critical elements that comprise the **GPI** -signal-the cleavage/attachment site and the COOH terminal hydrophobic domain-the former is responsible for the impaired activity of the VSG **GPI** signal in COS cells. To confirm this, we show that the VSG **GPI** signal can be converted to a viable signal for mammalian cells by altering the amino acid configuration at the cleavage/attachment site. We also show that when fused to the COOH terminus of hGH, the putative **GPI** signal from the **malaria** circumsporozoite (CS) protein produces low levels of **GPI**-anchored hGH, suggesting that the CS protein is indeed **GPI** linked, but that the CS protein **GPI** signal, like the VSG-signal, functions poorly in COS cells. The finding that the requirements for **GPI** attachment are similar but not identical in **parasitic** protozoa and mammalian cells may allow for the development of selective inhibitors of **GPI** -anchoring that might prove useful as antiparasite therapeutics.

L13 ANSWER 13 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1994:161129 BIOSIS
DN PREV199497174129
TI The requirements for **GPI**-attachment are similar but not identical in mammalian cells and **parasitic** protozoa.
AU Caras, I. W. (1); Moran, P.
CS (1) Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080 USA
SO Brazilian Journal of Medical and Biological Research, (1994) Vol. 27, No. 2, pp. 185-188.
ISSN: 0100-879X.
DT Article
LA English
AB To test whether the requirements for **GPI**-attachment are the same in mammalian cells and **parasitic** protozoa, we expressed the **GPI**-linked variant surface glycoprotein (VSG) of **Trypanosoma** brucei (T. brucei) in COS cells. Although large amounts of VSG were produced, only a small fraction became **GPI** -linked. This impaired processing is not due to the VSG ectodomain since replacement of the VSG **GPI**-signal with that of decay accelerating factor (DAF) produced **GPI**-linked VSG. Further, whereas fusion of the DAF **GPI**-signal to the COOH-terminus of human growth hormone (hGH) produces **GPI**-linked hGH, an analogous fusion using the VSG **GPI**-signal does not, indicating that the VSG **GPI**-signal functions poorly in mammalian cells. By constructing chimeric VSG-DAF **GPI**-signals and fusing them to the COOH-terminus of hGH, we show that of the two critical elements that comprise the **GPI**-signal - the cleavage/attachment site and the hydrophobic domain - the former is responsible for the impaired activity of the VSG **GPI**-signal in COS cells. To confirm this, we show that the VSG **GPI**-signal can be converted to a viable signal for mammalian cells by altering the amino acid configuration at the cleavage/attachment site. We also show that when fused to hGH, the putative **GPI**-signal from the **malaria** circumsporozoite (CS) protein produces low levels of **GPI**-anchored hGH, suggesting that the CS protein is indeed **GPI**-linked, but that the CS protein **GPI**-signal, like the VSG-signal, functions poorly in COS cells.

L13 ANSWER 14 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1992:390892 BIOSIS
DN BA94:63067
TI **PLASMODIUM**-FALCIPARUM AND **PLASMODIUM**-CHABAUDI CHARACTERIZATION OF GLYCOSYLPHOSPHATIDYLINOSITOL-DEGRADING ACTIVITIES.
AU BRAUN-BRETON C; BLISNICK T; BARBOT P; BUELOW R; PEREIRA DA SILVA L;

LANGSLEY G
CS UNIT EXP. PARASITOL., URA CNRS 146, DEP. IMMUNOL. INST. PASTEUR, 25 RUE DU DR. ROUX, 75015 PARIS, FR.
SO EXP PARASITOL, (1992) 74 (4), 452-462.
CODEN: EXPAAA. ISSN: 0014-4894.
FS BA; OLD
LA English
AB Merozoites of **malaria parasites** have a membrane-bound serine protease whose solubilization and subsequent activity depend on a **parasite**-derived glycosylphosphatidylinositol-phospholipase C (**GPI**-PLC). The GIP-degrading activities from both **Plasmodium** falciparum and Plasmoidum chabaudi have been characterized and partially purified by phenylboronate chromatography. They are membrane-bound, developmentally regulated, calcium-independent enzymes and as such they resemble **GPI**-PLC of **Trypanosoma** brucei. Furthermore, a T. brucei **GPI**-PLC-specific monoclonal antibody (mAT3) immunoprecipitates the plasmodial **GPI**-degrading activity. Thin-layer chromatography is suggestive of two activities: A **GPI**-PLC and a phospholipase A.

Braun-Breton et al

=> d his

(FILE 'HOME' ENTERED AT 14:46:32 ON 06 MAY 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 14:47:23 ON 06 MAY 2003
E SCHOFIELD LOUIS/AU
L1 9 S E1
L2 41 S E3
L3 26 S L2 AND GPI
L4 7 S L3 AND VACCIN?
L5 4 DUP REM L4 (3 DUPLICATES REMOVED)
L6 14 DUP REM L3 (12 DUPLICATES REMOVED)
L7 19791 S GPI OR INOSITOLGLYCAN DOMAIN OR INOSITOLGLYCAN
L8 1890 S L7 AND (PARASIT? OR PLASMODIUM)
L9 767 S L8 AND (TRYPANOSOMA OR LEISHMAINA OR TOXOPLASMA OR CANDIDA O
L10 281 DUP REM L9 (486 DUPLICATES REMOVED)
L11 12 S L10 AND VACCIN?
L12 45 S L9 AND MALARIA
L13 14 DUP REM L12 (31 DUPLICATES REMOVED)

=> s l9 and (lipid? or glycan)
L14 156 L9 AND (LIPID? OR GLYCAN)

=> s l14 and vaccin?
L15 3 L14 AND VACCIN?

=> d bib ab 1-3

L15 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:122678 BIOSIS
DN PREV200000122678
TI An early step of glycosylphosphatidyl-inositol anchor biosynthesis is abolished in lepidopteran insect cells following baculovirus infection.
AU Azzouz, Nahid; Kedees, Mamdouh H.; Gerold, Peter; Becker, Stephan; Dubremetz, Jean-Francois; Klenk, Hans-Dieter; Eckert, Volker; Schwarz, Ralph T. (1)
CS (1) Med. Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg Germany
SO Glycobiology, (Feb., 2000) Vol. 10, No. 2, pp. 177-183.
ISSN: 0959-6658.

modification of recombinant proteins expressed in insect cells, as compared to the native proteins expressed in **parasites**, and the possible implications for **vaccine** development are discussed.

L15 ANSWER 3 OF 3 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-271259 [23] WPIDS
DNC C2000-082769
TI Inducing immune response against a microorganism, useful particularly for treating or preventing malaria, by administering an **inositolglycan domain** of glycosylphosphatidylinositol.
DC B04 D16
IN SCHOFIELD, L
PA (HALL-N) HALL INST MEDICAL RES WALTER & ELIZA
CYC 90
PI WO 2000015254 A1 20000323 (200023)* EN 100p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9958420 A 20000403 (200034)
EP 1113815 A1 20010711 (200140) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT WO 2000015254 A1 WO 1999-AU770 19990914; AU 9958420 A AU 1999-58420 19990914; EP 1113815 A1 EP 1999-945777 19990914, WO 1999-AU770 19990914
FDT AU 9958420 A Based on WO 200015254; EP 1113815 A1 Based on WO 200015254
PRAI AU 1998-5893 19980914
AB WO 200015254 A UPAB: 20000516
NOVELTY - Method of eliciting or inducing, in a mammal, an immune response against a microorganism comprises administering a composition (A), containing a compound (I) that induces a response to the **inositolglycan domain** of a **GPI** (glycosylphosphatidylinositol), but not to the **lipid** domain of **GPI**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of therapeutically or prophylactically treating microorganism infection by administering (A);

(2) the composition (A);

(3) a **vaccine** or pharmaceutical composition containing (I) and at least one carrier and/or diluent;

(4) an antibody (Ab) directed to the **inositolglycan domain** of **GPI**, which does not interact with the **lipid** domain;

(5) a pharmaceutical composition containing Ab and at least one carrier and/or diluent; and

(6) a method of inhibiting, halting or delaying mammalian disease onset or progression, caused by microorganism infection, by administering Ab.

ACTIVITY - Antimicrobial; antifungal; protozoacide. Mice were infected with 1 million **Plasmodium** bergheri ANKA, then after 4 days treated daily (for 7 days) with 0.1 mu g/day (intraperitoneally) of the monoclonal antibody 1D12 (raised in mice against a conjugate of **Plasmodium GPI glycan** domain and ovalbumin).
All untreated controls died of cerebral malaria 6-8 days after infection, but all treated animals were still alive after 14 days.

MECHANISM OF ACTION - **Vaccine**. **GPI** is a toxin that regulates host cell function and gene expression, resulting in activation of transcription factors that upregulate expression of proinflammatory molecules. (I) reduce both the toxic and immunosuppressive effects of **GPI** or it kills microbial **parasites**

directly.

USE - (A) is used to treat and/or prevent microbial infections, especially **Plasmodium** infections, and malaria (claimed), but also infection by **Trypanosoma**, Leishmania, **Toxoplasma** and **Candida**. Antibodies (Ab) directed against the **inositolglycan domain** can also be used to treat or prevent infections, and as immunoassay reagents for detecting the **GPI** domain, e.g. for diagnosis of **parasitic** infection, autoimmune and degenerative diseases, and somatic acquired genetic defects, or for monitoring therapy, and for affinity purification of recombinant **GPI**.

ADVANTAGE - Fragments of **GPI** that lack the **lipid** domain induce a protective response, although those that contain the **lipid** domain do not, and produce an immunoglobulin M that exacerbates the disease.

Dwg.0/12

=> dup rem l14
PROCESSING COMPLETED FOR L14
L16 66 DUP REM L14 (90 DUPLICATES REMOVED)

=> d bib ab 1-66

L16 ANSWER 1 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2003:191241 BIOSIS
DN PREV200300191241
TI Alkylacylglycerolipid domain of **GPI** molecules of Leishmania is responsible for inhibition of PKC-mediated c-fos expression.
AU Chawla, Mamta; Vishwakarma, Ram A. (1)
CS (1) Bio-organic Chemistry Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, 110 067, India: ram@nii.res.in India
SO Journal of Lipid Research, (March 2003, 2003) Vol. 44, No. 3, pp. 594-600. print.
ISSN: 0022-2275.
DT Article
LA English
AB Glycosylphosphatidylinositols (GPIs) are the most abundant molecules present in the membranes of the **parasitic** protozoa Leishmania responsible for multiple forms of leishmaniasis. Among the prominent biological activities displayed by the major Leishmania GPIs (lipophosphoglycan (LPG) and glycoinositolphospholipids (GIPLs)) is the inhibition of macrophage functions such as the protein kinase C (PKC)-dependent signaling pathway. The bioactivity of Leishmania GPIs is in contrast to **Trypanosoma** brucei and **Plasmodium** falciparum GPIs, which activate the macrophage functions. To address the question as to which structural domain of Leishmania GPIs is responsible for dramatic down-regulation of PKC-dependent transient c-fos expression, the chemically synthesized defined alkylacylglycerolipids domain of corresponding GPIs, and LPG and GIPLs isolated from Leishmania donovani, were evaluated for inhibition of PKC and c-fos expression in macrophages. The results presented here demonstrate that the unusual **lipid** domain of Leishmania GPIs is primarily responsible for inhibition of PKC-dependent transient c-fos expression.

L16 ANSWER 2 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:182231 BIOSIS
DN PREV200300182231
TI A novel sialylated and galactofuranose-containing O-linked **glycan**, Neu5Acalpha2fwdarw3Galpbeta1fwdarw6(Galfbeta1fwdarw4)GlcNAc, is expressed on the sialoglycoprotein of **Trypanosoma** cruzi Dm28c.
AU Agrellos, Orlando A.; Jones, Christopher; Todeschini, Adriane R.;

Previato, Jose O.; Mendonca-Previato, Lucia (1)
CS (1) Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Ilha do Fundao, 21 944 970, Rio de Janeiro, RJ, Brazil: luciamp@biof.ufrj.br Brazil
SO Molecular & Biochemical Parasitology, (January 2003, 2003) Vol. 126, No. 1, pp. 93-96. print.
ISSN: 0166-6851.
DT Article
LA English

L16 ANSWER 3 OF 66 MEDLINE DUPLICATE 2
AN 2002449517 MEDLINE
DN 22118156 PubMed ID: 12121418
TI Accumulation of a **GPI**-anchored protein at the cell surface requires sorting at multiple intracellular levels.
AU Grunfelder Christoph G; Engstler Markus; Weise Frank; Schwarz Heinz; Stierhof York-Dieter; Boshart Michael; Overath Peter
CS Max-Planck-Institut fur Biologie, Abteilung Membranbiochemie, Correnssstrasse 38, D-72076 Tubingen, Germany.
SO TRAFFIC, (2002 Aug) 3 (8) 547-59.
Journal code: 100939340. ISSN: 1398-9219.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200302
ED Entered STN: 20020906
Last Updated on STN: 20030227
Entered Medline: 20030226
AB Proteins modified by glycosylphosphatidylinositol membrane anchors have become popular for investigating the role of membrane **lipid** microdomains in cellular sorting processes. To this end, trypanosomatids offer the advantage that they express these molecules in high abundance. The **parasitic** protozoan **Trypanosoma** brucei is covered by a dense and nearly homogeneous coat composed of a glycosylphosphatidylinositol-anchored protein, the variant surface glycoprotein, which is essential for survival of the **parasite** in the mammalian blood. Therefore, T. brucei must possess mechanisms to selectively and efficiently deliver variant surface glycoprotein to the cell surface. In this study, we have quantified the steady-state distribution of variant surface glycoprotein by differential biotinylation, by fluorescence microscopy and by immunoelectron microscopy on high-pressure frozen and freeze-substituted samples. These three techniques provide very similar estimates of the fraction of variant surface glycoprotein located on the cell surface, on average 89.4%. The intracellular variant surface glycoprotein (10.6%) is predominantly located in the endosomal compartment (75%), while 25% are associated with the endoplasmic reticulum, Golgi apparatus and lysosomes. The density of variant surface glycoprotein in the plasma membrane including the membrane of the flagellar pocket, the only site for endo- and exocytosis in this organism, is 48-52 times higher than the density in endoplasmic reticulum membranes. The relative densities of the Golgi complex and of the endosomes are 2.7 and 10.8, respectively, compared to the endoplasmic reticulum. This data set provides the basis for an analysis of the dynamics of sorting. Depending on the intracellular itinerary of newly formed variant surface glycoprotein, the high surface density is achieved in two (endoplasmic reticulum --> Golgi complex --> cell surface) or three enrichment steps (endoplasmic reticulum --> Golgi complex --> endosomes --> cell surface), suggesting sorting between several membrane compartments.

L16 ANSWER 4 OF 66 CABA COPYRIGHT 2003 CABI
AN 2001:65290 CABA

DN 20013047824
TI Requirement of mitogen-activated protein kinases and I kappa B phosphorylation for induction of proinflammatory cytokines synthesis by macrophages indicates functional similarity of receptors triggered by glycosylphosphatidylinositol anchors from **parasitic** protozoa and bacterial lipopolysaccharide
AU Ropert, C.; Almeida, I. C.; Closel, M.; Travassos, L. R.; Ferguson, M. A. J.; Cohen, P.; Gazzinelli, R. T.
CS Department of Biochemistry and Immunology, Rene Rachou Research Center-Fundacao Oswaldo Cruz, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
SO Journal of Immunology, (2001) Vol. 166, No. 5, pp. 3423-3431. 62 ref. ISSN: 0022-1767
DT Journal
LA English
AB The present study evaluated the ability of **GPI**-anchored mucin-like glycoproteins purified from **Trypanosoma** cruzi trypomastigotes (tGPI-mucin) to trigger phosphorylation of different mitogen-activated protein kinases (MAPKs) and related transcription factors in inflammatory macrophages. Kinetic experiments showed that the peak of extracellular signal-related kinase (ERK)-1/ERK-2, stress-activated protein kinase (SAPK) kinase-1/mitogen-activated protein kinase (MAPK) kinase-4, and p38/SAPK-2, phosphorylation occurs between 15 and 30 minutes after macrophage-stimulation with tGPI-mucin or **GPI** anchors highly purified from tGPI-mucins (tGPI). The use of the specific inhibitors of ERK-1/ERK-2 (PD 98059) and p38/SAPK-2 (SB 203580) phosphorylation indicated the role of MAPKs, with possible involvement of cAMP response element binding protein, in triggering TNF- alpha and IL-12 synthesis by IFN- gamma -primed-macrophages exposed to tGPI or tGPI-mucin. In addition, tGPI-mucin and tGPI were able to induce phosphorylation of I kappa B, and the use of SN50 peptide, an inhibitor of NF- kappa B translocation, resulted in 70% of TNF- alpha synthesis by macrophages exposed to tGPI-mucin. The similarity of patterns of MAPK and I kappa B phosphorylation, the concentration of drugs required to inhibit cytokine synthesis, as well as the cross-tolerance exhibited by macrophages exposed to tGPI, tGPI-mucin, or bacterial LPS, suggest that receptors with the same functional properties are triggered by these different microbial glycoconjugates.

L16 ANSWER 5 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2001:384606 BIOSIS
DN PREV200100384606
TI Specificity of GlcNAc-PI de-N-acetylase of **GPI** biosynthesis and synthesis of **parasite**-specific suicide substrate inhibitors.
AU Smith, Terry K.; Crossman, Arthur; Borissow, Charles N.; Paterson, Michael J.; Dix, Alex; Brimacombe, John S.; Ferguson, Michael A. J. (1)
CS (1) Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee, DD1 5EH: m.a.j.ferguson@dundee.ac.uk UK
SO EMBO (European Molecular Biology Organization) Journal, (July 2, 2001) Vol. 20, No. 13, pp. 3322-3332. print. ISSN: 0261-4189.
DT Article
LA English
SL English
AB The substrate specificities of **Trypanosoma** brucei and human (HeLa) GlcNAc-PI de-N-acetylases were determined using 24 substrate analogues. The results show the following. (i) The de-N-acetylases show little specificity for the **lipid** moiety of GlcNAc-PI. (ii) The 3'-OH group of the GlcNAc residue is essential for substrate recognition whereas the 6'-OH group is dispensable and the 4'-OH, while not required for recognition, cannot be epimerized or substituted. (iii) The

parasite enzyme can act on analogues containing betaGlcNAc or aromatic N-acyl groups, whereas the human enzyme cannot. (iv) Three GlcNR-PI analogues are de-N-acetylase inhibitors, one of which is a suicide inhibitor. (v) The suicide inhibitor most likely forms a carbamate or thiocarbamate ester to an active site hydroxy-amino acid or Cys or residue such that inhibition is reversed by certain nucleophiles. These and previous results were used to design two potent (IC50=8 nM) **parasite**-specific suicide substrate inhibitors. These are potential lead compounds for the development of anti-protozoan **parasite** drugs.

L16 ANSWER 6 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001426408 EMBASE
TI Multiple procyclin isoforms are expressed differentially during the development of insect forms of **Trypanosoma** brucei.
AU Vassella E.; Acosta-Serrano A.; Studer E.; Lee S.H.; Englund P.T.; Roditi I.
CS I. Roditi, Institut fur Zellbiologie, Universitat Bern, CH-3012 Bern, Switzerland. isabel.roditi@izb.unibe.ch
SO Journal of Molecular Biology, (28 Sep 2001) 312/4 (597-607).
Refs: 33
ISSN: 0022-2836 CODEN: JMOBAK
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Transmission of **Trypanosoma** brucei by the tsetse fly entails several rounds of differentiation as the **parasite** migrates through the digestive tract to the salivary glands of its vector. Differentiation of the blood-stream to the procyclic form in the fly midgut is accompanied by the synthesis of a new coat consisting of EP and GPEET procyclins. There are three closely related EP isoforms, two of which (EP1 and EP3) contain N-glycans. To identify the individual EP isoforms that are expressed early during synchronous differentiation in vitro, we exploited the selective extraction of **GPI**-anchored proteins and mass spectrometry. Unexpectedly, we found that GPEET and all isoforms of EP were coexpressed for a few hours at the onset of differentiation. At this time, the majority of EP1 and EP3 molecules were already glycosylated. Within 24 hours, GPEET became the major surface component, to be replaced in turn by glycosylated forms of EP, principally EP1, at a later phase of development. Transient transfection experiments using reporter genes revealed that each procyclin 3' untranslated region contributes to differential expression as the procyclic form develops. We postulate that programmed expression of other procyclin species will accompany further rounds of differentiation, enabling the **parasite** to progress through the fly. .COPYRGT. 2001 Academic Press.

L16 ANSWER 7 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001361802 EMBASE
TI Proinflammatory activity of glycosylphosphatidylinositol anchors derived from **Trypanosoma** cruzi: Structural and functional analyses.
AU Almeida I.C.; Gazzinelli R.T.
CS Dr. I.C. Almeida, Department of Parasitology, Institute of Biomedical Sciences, University of Sao Paulo, Av. Prof. Lineu Prestes 1374, Sao Paulo, SP 05508-900, Brazil. ialmeida@icb.usp.br
SO Journal of Leukocyte Biology, (2001) 70/4 (467-477).
Refs: 96
ISSN: 0741-5400 CODEN: JLBIE7
CY United States
DT Journal; General Review
FS 004 Microbiology
026 Immunology, Serology and Transplantation

infections.

L16 ANSWER 9 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2001:424346 BIOSIS
DN PREV200100424346
TI Fatty acid remodeling of glycosyl phosphatidylinositol anchors in **Trypanosoma** brucei: Incorporation of fatty acids other than myristate.
AU Morita, Yasu S.; Englund, Paul T. (1)
CS (1) Department of Biological Chemistry, Johns Hopkins Medical School, 725 N. Wolfe St., Baltimore, MD, 21205: penglund@jhmi.edu USA
SO Molecular and Biochemical Parasitology, (July, 2001) Vol. 115, No. 2, pp. 157-164. print.
ISSN: 0166-6851.
DT Article
LA English
SL English
AB **Trypanosoma** brucei is the protozoan **parasite** that causes African sleeping sickness. Its surface is packed with 107 copies of the glycosyl phosphatidylinositol (**GPI**)-anchored variant surface glycoprotein (VSG). This **GPI** anchor is unusual in that it contains two myristates (14:0) in its **lipid** moiety. This fatty acid specificity is achieved through myristoylation of the **GPI** precursor, and the acyltransferases involved in the **GPI** remodeling were presumed to be specific for myristate. However, their specificity had never been fully evaluated. Here we found as expected that the remodeling acyltransferases completely excluded palmitate (16:0) and stearate (18:0) in a cell-free fatty acid remodeling system. In contrast, we found surprisingly that one of these enzymes was permissive to shorter fatty acids such as laurate (12:0) and octanoate (8:0). However, the rates of incorporation of shorter fatty acids were lower than that of myristate at low substrate concentration. Since shorter fatty acids are virtually absent in the **parasite** and in the host bloodstream, it is unlikely that shorter fatty acids compete effectively with myristate as remodeling substrates under physiological conditions. Even if they were present in small quantities, a recently identified specialized fatty acid synthetase efficiently elongates shorter fatty acids to myristate prior to incorporation into GPIs (Morita et al., Science 288 (2000) 140-3.). Therefore, even though a remodeling acyltransferase is permissive with regard to substrate chain length, the myristate specificity in **GPI** anchors is very high.

L16 ANSWER 10 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 2001:150915 BIOSIS
DN PREV200100150915
TI **GPI**-anchored proteins and glycoconjugates segregate into **lipid** rafts in Kinetoplastida.
AU Denny, Paul W. (1); Field, Mark C.; Smith, Deborah F.
CS (1) Wellcome Trust Laboratories for Molecular Parasitology, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 1AZ: p.w.denny@ic.ac.uk UK
SO FEBS Letters, (23 February, 2001) Vol. 491, No. 1-2, pp. 148-153. print.
ISSN: 0014-5793.
DT Article
LA English
SL English
AB The plasma membranes of the divergent eukaryotic **parasites**, Leishmania and **Trypanosoma**, are highly specialised, with a thick coat of glycoconjugates and glycoproteins playing a central role in virulence. Unusually, the majority of these surface macro-molecules are attached to the plasma membrane via a glycosylphosphatidylinositol (

GPI) anchor. In mammalian cells and yeast, many **GPI**-anchored molecules associate with sphingolipid and cholesterol-rich detergent-resistant membranes, known as **lipid** rafts. Here we show that **GPI**-anchored **parasite** macro-molecules (but not the dual acylated Leishmania surface protein (hydrophilic acylated surface protein) or a subset of the **GPI**-anchored glycoinositol phospholipid glycolipids) are enriched in a sphingolipid/sterol-rich fraction resistant to cold detergent extraction. This observation is consistent with the presence of functional **lipid** rafts in these ancient, highly polarised organisms.

L16 ANSWER 11 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2001201265 EMBASE
TI The mucin-like glycoprotein super-family of **Trypanosoma** cruzi: Structure and biological roles.
AU Acosta-Serrano A.; Almeida I.C.; Freitas-Junior L.H.; Yoshida N.; Schenkman S.
CS A. Acosta-Serrano, School of Life Science, Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, United Kingdom. a.f.acostaserrano@dundee.ac.uk
SO Molecular and Biochemical Parasitology, (2001) 114/2 (143-150). Refs: 67
ISSN: 0166-6851 CODEN: MBIPDP
PUI S 0166-6851(01)00245-6
CY Netherlands
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB **Trypanosoma** cruzi expresses at its surface large amounts of mucin-like glycoproteins. The T. cruzi mucins (TcMUC), a group of highly glycosylated **GPI**-anchored proteins rich in Thr, Ser, and Pro residues, are expressed in high copy numbers in both insect and mammalian stages of the **parasite**. These molecules are encoded by a multigene family and contain a unique type of glycosylation consisting of several sialylated O-glycans linked to the protein backbone via N-acetylglucosamine residues. The TcMUC are important because of their role in host cell invasion and the ability to induce secretion of proinflammatory cytokines and nitric oxide in activated macrophages. The TcMUC are also significant in being the major substrate for the cell surface trans-sialidase. In this review, we summarize the recent knowledge on the molecular structure and function of this family of T. cruzi glycoproteins. .COPYRGT. 2001 Elsevier Science B.V.

L16 ANSWER 12 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 2001:478824 BIOSIS
DN PREV200100478824
TI Two glycoforms are present in the **GPI**-membrane anchor of the surface antigen 1 (P30) of **Toxoplasma** gondii.
AU Zinecker, Christina F.; Striepen, Boris; Geyer, Hildegard; Geyer, Rudolf; Dubremetz, Jean-Francois; Schwarz, Ralph T. (1)
CS (1) Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, D-35037, Marburg: schwarz@mailer.uni-marburg.de Germany
SO Molecular & Biochemical Parasitology, (3 September, 2001) Vol. 116, No. 2, pp. 127-135. print.
ISSN: 0166-6851.
DT Article
LA English
SL English
AB SAG1 (P30) is the major surface protein of the **Toxoplasma** gondii tachyzoite, the life cycle stage associated with the acute phase of

infection. The protein is inserted into the **parasite**'s plasma membrane by a glycosyl-phosphatidylinositol anchor, a modification that is present on all T. gondii surface proteins characterized so far. Here we describe a detailed structural analysis of this anchor. **GPI** anchor peptides were isolated from (3H)glucosamine labeled purified P30 by protease digestion and phase partitioning. Neutral glycans were prepared from this material by dephosphorylation and deamination. Two glycoforms were characterized by gel filtration and high performance ion exchange chromatography in combination with exoglycosidase treatment. Both forms were shown to carry an N-acetylgalactosamine bound to the first mannose of the conserved three-mannosyl core. **Glycan** B carries an additional terminal hexose linked to GalNAc. To identify the nature of this hexose, bulk anchor peptide was prepared and glycans were purified by aminopropyl-HPLC. Highly purified glycans were subjected to MALDI-TOF-MS and, after derivatization, to FAB-MS and methylation linkage analysis. The structures of the two anchors found on SAG1 were determined to be: Man-alpha1,2-Man-alpha1,6-Man-(GalNAc-beta1,4-)-alpha1,4-GlcN-PI and Man-alpha1,2-Man-alpha1,6-Man (Glc-alpha1,4-GalNAc-beta1,4-)-alpha1,4-GlcN-PI. Comparison of these structures with free **GPI** glycolipid precursors characterized in T. gondii suggests that core modification of the anchor takes place prior to transfer to the protein.

L16 ANSWER 13 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:330265 BIOSIS
DN PREV200100330265
TI Glycoconjugate structures of **parasitic** protozoa.
AU Guha-Niyogi, Anuradha; Sullivan, Deborah R.; Turco, Salvatore J. (1)
CS (1) Department of Molecular and Cellular Biochemistry, University of Kentucky Medical Center, Lexington, KY, 40536 USA
SO Glycobiology, (April, 2001) Vol. 11, No. 4, pp. 45R-59R. print. ISSN: 0959-6658.
DT General Review
LA English
SL English
AB Glycoconjugates are abundant and ubiquitious on the surface of many protozoan **parasites**. Their tremendous diversity has implicated their critical importance in the life cycle of these organisms. This review highlights our current knowledge of the major glycoconjugates, with particular emphasis on their structures, of representative protozoan **parasites**, including Leishmania, **Trypanosoma**, Giardia, Plasmodia, and others.

L16 ANSWER 14 OF 66 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-271444 [23] WPIDS
DNN N2000-203228 DNC C2000-082936
TI In vivo down-regulation of osteoprotegerin ligand (OPGL) activity used to treat, prevent and ameliorate osteoporosis.
DC B04 D16 S03
IN HAANING, J; HALKIER, T
PA (MEBI-N) M & E BIOTECH AS; (PHAR-N) PHARMEXA AS
CYC 89
PI WO 2000015807 A1 20000323 (200023)* EN 110p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9956173 A 20000403 (200034)
NO 2001001304 A 20010515 (200137)
EP 1114166 A1 20010711 (200140) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SI

CZ 2001000789 A3 20010815 (200157)
CN 1318105 A 20011017 (200213)
SK 2001000306 A3 20020205 (200213)
KR 2001085807 A 20010907 (200218)
HU 2001003578 A2 20020128 (200222)
ZA 2001002131 A 20020828 (200264) 153p
JP 2002525060 W 20020813 (200267) 104p
AU 754971 B 20021128 (200306)

ADT WO 2000015807 A1 WO 1999-DK481 19990913; AU 9956173 A AU 1999-56173 19990913; NO 2001001304 A WO 1999-DK481 19990913, NO 2001-1304 20010314; EP 1114166 A1 EP 1999-942778 19990913, WO 1999-DK481 19990913; CZ 2001000789 A3 WO 1999-DK481 19990913, CZ 2001-789 19990913; CN 1318105 A CN 1999-810872 19990913; SK 2001000306 A3 WO 1999-DK481 19990913, SK 2001-306 19990913; KR 2001085807 A KR 2001-703379 20010315; HU 2001003578 A2 WO 1999-DK481 19990913, HU 2001-3578 19990913; ZA 2001002131 A ZA 2001-2131 20010314; JP 2002525060 W WO 1999-DK481 19990913, JP 2000-570334 19990913; AU 754971 B AU 1999-56173 19990913

FDT AU 9956173 A Based on WO 200015807; EP 1114166 A1 Based on WO 200015807; CZ 2001000789 A3 Based on WO 200015807; SK 2001000306 A3 Based on WO 200015807; HU 2001003578 A2 Based on WO 200015807; JP 2002525060 W Based on WO 200015807; AU 754971 B Previous Publ. AU 9956173, Based on WO 200015807

PRAI US 1998-102896P 19981002; DK 1998-1164 19980915

AB WO 200015807 A UPAB: 20021105

NOVELTY - In vivo down-regulation of osteoprotegerin ligand (OPGL) activity in an animal, including a human, comprising presentation of at least one OPGL polypeptide or subsequence, and/or at least one OPGL analog to the animal to induce an immune response, is new.

DETAILED DESCRIPTION - In vivo down-regulation of osteoprotegerin ligand (OPGL) activity in an animal, including a human, comprises effecting presentation to the animal's immune system of:

(a) at least one OPGL polypeptide or subsequence which has been formulated so that immunization of the animal with the OPGL polypeptide or subsequence induces production of antibodies against the OPGL polypeptide; and/or

(b) at least one OPGL analog where at least one modification in the OPGL amino acid sequence is introduced which has a result that immunization of the animal with the analog induces production of antibodies against the OPGL polypeptide.

INDEPENDENT CLAIMS are also included for the following:

(1) an OPGL analog which is derived from an animal OPGL polypeptide where a modification is introduced which has a result that immunization of the animal with the analog induces production of antibodies against the OPGL polypeptide;

(2) a nucleic acid fragment which encodes the OPGL analog of (1);

(3) a vector carrying the nucleic acid fragment of (2);

(4) a stable cell line which carries the vector of (3) expressing the nucleic acid fragment of (2) and which optionally secretes or carries the OPGL analog of (1);

(5) a method for the preparation of the cell in (4) comprising transforming a host cell with the nucleic acid fragment in (2) or the vector of (3);

(6) a method for the identification of a modified OPGL polypeptide which is capable of inducing antibodies against unmodified OPGL in an animal species where the unmodified OPGL polypeptide is a self-protein comprising:

(a) preparing, by peptide synthesis or genetic engineering techniques, a set of mutually distinct modified OPGL polypeptides where amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an OPGL polypeptide of the animal species giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal species, or preparing a set of nucleic acid fragments encoding the set of mutually distinct modified OPGL

polypeptides;

(b) testing members of the set of modified OPGL polypeptides or nucleic acid fragments for their ability to induce production of antibodies by the animal species against the unmodified OPGL; and

(c) identifying and optionally isolating the member(s) of the set of modified OPGL polypeptides which significantly induces antibody production against unmodified OPGL in the species or identifying and optionally isolating the polypeptide expression products encoded by members of the set of nucleic acid fragments which significantly induces antibody production against unmodified OPGL in the animal species;

(7) a method for the preparation of an immunogenic composition comprising at least one modified OPGL polypeptide which is capable of inducing antibodies against unmodified OPGL in an animal species where the unmodified OPGL polypeptide is a self-protein comprising:

(a) preparing, by peptide synthesis or genetic engineering techniques, a set of mutually distinct modified OPGL polypeptides where amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an OPGL polypeptide of the animal species giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal;

(b) testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified OPGL; and

(c) admixing the member(s) of the set which significantly induces antibody production in the animal species which are reactive with OPGL with a pharmaceutically and immunologically acceptable carrier and/or vehicle.

ACTIVITY - Osteopathic; immunostimulatory.

No biological data.

MECHANISM OF ACTION - Down-regulation of osteoprotegerin ligand (OPGL) activity.

USE - The method and polypeptide is useful for treating, preventing and ameliorating osteoporosis or other diseases or conditions characterized by excessive bone resorption. The OPGL or subsequence is useful for down-regulating OPGL activity in an animal.

Dwg.0/0

L16 ANSWER 15 OF 66 WPIDS (C) 2003 THOMSON DERWENT
AN 2000-271259 [23] WPIDS
DNC C2000-082769
TI Inducing immune response against a microorganism, useful particularly for treating or preventing malaria, by administering an **inositolglycan domain** of glycosylphosphatidylinositol.
DC B04 D16
IN SCHOFIELD, L
PA (HALL-N) HALL INST MEDICAL RES WALTER & ELIZA
CYC 90
PI WO 2000015254 A1 20000323 (200023)* EN 100p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 9958420 A 20000403 (200034)
EP 1113815 A1 20010711 (200140) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
ADT WO 2000015254 A1 WO 1999-AU770 19990914; AU 9958420 A AU 1999-58420 19990914; EP 1113815 A1 EP 1999-945777 19990914, WO 1999-AU770 19990914
FDT AU 9958420 A Based on WO 200015254; EP 1113815 A1 Based on WO 200015254
PRAI AU 1998-5893 19980914
AB WO 200015254 A UPAB: 20000516
NOVELTY - Method of eliciting or inducing, in a mammal, an immune response

against a microorganism comprises administering a composition (A), containing a compound (I) that induces a response to the **inositolglycan domain** of a **GPI** (glycosylphosphatidylinositol), but not to the **lipid** domain of **GPI**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of therapeutically or prophylactically treating microorganism infection by administering (A);

(2) the composition (A);

(3) a vaccine or pharmaceutical composition containing (I) and at least one carrier and/or diluent;

(4) an antibody (Ab) directed to the **inositolglycan domain** of **GPI**, which does not interact with the **lipid** domain;

(5) a pharmaceutical composition containing Ab and at least one carrier and/or diluent; and

(6) a method of inhibiting, halting or delaying mammalian disease onset or progression, caused by microorganism infection, by administering Ab.

ACTIVITY - Antimicrobial; antifungal; protozoacide. Mice were infected with 1 million **Plasmodium** bergheri ANKA, then after 4 days treated daily (for 7 days) with 0.1 mu g/day (intraperitoneally) of the monoclonal antibody 1D12 (raised in mice against a conjugate of **Plasmodium GPI glycan** domain and ovalbumin). All untreated controls died of cerebral malaria 6-8 days after infection, but all treated animals were still alive after 14 days.

MECHANISM OF ACTION - Vaccine. **GPI** is a toxin that regulates host cell function and gene expression, resulting in activation of transcription factors that upregulate expression of proinflammatory molecules. (I) reduce both the toxic and immunosuppressive effects of **GPI** or it kills microbial **parasites** directly.

USE - (A) is used to treat and/or prevent microbial infections, especially **Plasmodium** infections, and malaria (claimed), but also infection by **Trypanosoma**, Leishmania, **Toxoplasma** and **Candida**. Antibodies (Ab) directed against the **inositolglycan domain** can also be used to treat or prevent infections, and as immunoassay reagents for detecting the **GPI** domain, e.g. for diagnosis of **parasitic** infection, autoimmune and degenerative diseases, and somatic acquired genetic defects, or for monitoring therapy, and for affinity purification of recombinant **GPI**.

ADVANTAGE - Fragments of **GPI** that lack the **lipid** domain induce a protective response, although those that contain the **lipid** domain do not, and produce an immunoglobulin M that exacerbates the disease.

Dwg.0/12

L16 ANSWER 16 OF 66 CAPLUS COPYRIGHT 2003 ACS
AN 2000:234769 CAPLUS
DN 133:70556
TI Specific inhibition of an .alpha.-galactosyltransferase from **Trypanosoma** brucei by synthetic substrate analogues
AU Kolb, Volker; Amann, Franz; Schmidt, Richard R.; Duszenko, Michael
CS Physiologisch-chemisches Institut der Universitat Tubingen, Tubingen, D-72076, Germany
SO Glycoconjugate Journal (2000), Volume Date 1999, 16(9), 537-544
CODEN: GLJOEW; ISSN: 0282-0080
PB Kluwer Academic Publishers
DT Journal
LA English
AB Since the .alpha.-D-galactose-(1.fwdarw.3)-D-galactose epitope has been identified to be the major target in the process of hyperacute rejection

LA English
SL English
AB The expression of recombinant proteins in their native state has become a prerequisite for a variety of functional and structural studies, as well as vaccine development. Many biochemical properties and functions of proteins are dependent on or reside in posttranslational modifications, such as glycosylation. The baculovirus system has increasingly become the system of choice due to it capabilities of performing posttranslational modifications and usually high yields of recombinant proteins. The **Toxoplasma** gondii surface antigen SAG1 was used as a model for a glycosylphosphatidyl-inositol (**GPI**)-anchored protein and expressed in insect cells using the baculovirus system. We show that the T. gondii SAG1 surface antigen expressed in this system was not modified by a **GPI**-anchor. In vitro and in vivo studies demonstrate that uninfected insect cells are able to produce **GPI**-precursors and to transfer a mature **GPI**-anchor to nascent proteins. These cells however are not capable to produce **GPI**-precursors following infection. We also show that the biosynthesis of the early **GPI** intermediate GlcNH2-PI is blocked in baculovirus-infected H5 cells, thus preventing the subsequent mannosylation steps for the synthesis of the conserved **GPI**-core-**glycan**. We therefore conclude that the baculovirus system is not appropriate for the expression of **GPI**-anchored proteins.

L16 ANSWER 19 OF 66 CABA COPYRIGHT 2003 CABI
AN 2000:40742 CABA
DN 20000805099
TI Structural analysis of free and protein-bound glycosyl-phosphatidylinositols of Neospora caninum
AU Schares, G.; Zinecker, C. F.; Schmidt, J.; Azzouz, N.; Conraths, F. J.; Gerold, P.; Schwarz, R. T.
CS Bundesforschungsanstalt fur Viruskrankheiten der Tiere, Institut fur epidemiologische Diagnostik, 16868 Wusterhausen, Germany.
SO Molecular and Biochemical Parasitology, (2000) Vol. 105, No. 1, pp. 155-161. 18 ref.
ISSN: 0166-6851
DT Journal
LA English
AB The structure of free and protein-bound glycosylphosphatidylinositols (GPIs) of Neospora caninum was elucidated for the first time. The results indicate that the **GPI** anchor of the p38 surface antigen of N. caninum tachyzoites is modified by a fatty acid bound to the inositol and has the evolutionary conserved trimannosyl core **glycan**. This is in contrast to findings on GPIs from the closely-related **Toxoplasma** gondii.

L16 ANSWER 20 OF 66 MEDLINE DUPLICATE 8
AN 2000217224 MEDLINE
DN 20217224 PubMed ID: 10753118
TI Specialized fatty acid synthesis in African trypanosomes: myristate for **GPI** anchors.
AU Morita Y S; Paul K S; Englund P T
CS Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205, USA.
NC AI21334 (NIAID)
SO SCIENCE, (2000 Apr 7) 288 (5463) 140-3.
Journal code: 0404511. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200004
ED Entered STN: 20000421

Last Updated on STN: 20000421
Entered Medline: 20000413

AB African trypanosomes, the cause of sleeping sickness, need massive amounts of myristate to remodel glycosyl phosphatidylinositol (**GPI**) anchors on their surface glycoproteins. However, it has been believed that the **parasite** is unable to synthesize any fatty acids, and myristate is not abundant in the hosts' bloodstreams. Thus, it has been unclear how trypanosomes meet their myristate requirement. Here we found that they could indeed synthesize fatty acids. The synthetic pathway was unique in that the major product, myristate, was preferentially incorporated into GPIs and not into other **lipids**. The antibiotic thiolactomycin inhibited myristate synthesis and killed the **parasite**, making this pathway a potential chemotherapeutic target.

L16 ANSWER 21 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2000059865 EMBASE
TI Processing and localisation of a **GPI**-anchored **Plasmodium** falciparum surface protein expressed by the baculovirus system.
AU Kedees M.H.; Gerold P.; Azzouz N.; Blaschke T.; Shams-Eldin H.; Muhlberger E.; Holder A.A.; Klenk H.-D.; Schwarz R.T.; Eckert V.
CS Prof. R.T. Schwarz, Zent. Hygiene Medizin. Mikrobiol., Philipps-Universitat Marburg, Robert-Koch-Strasse 17, D-35037 Marburg, Germany. schwarz@mailer.uni-marburg.de
SO European Journal of Cell Biology, (2000) 79/1 (52-61).
Refs: 69
ISSN: 0171-9335 CODEN: EJCBDN
CY Germany
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB We describe the expression, in insect cells using the baculovirus system, of two protein fragments derived from the C-terminus of merozoite surface protein 1 (MSP-1) of the human malaria **parasite** **Plasmodium** falciparum, and their glycosylation and intracellular location. The transport and intracellular localisation of the intact C-terminal MSP-1 fragment, modified by addition of a signal sequence for secretion, was compared with that of a similar control protein in which translation of the **GPI**-cleavage/attachment site was abolished by insertion of a stop codon into the DNA sequence. Both proteins could only be detected intracellularly, most likely in the endoplasmic reticulum. This lack of transport to the cell surface or beyond, was confirmed for both proteins by immunofluorescence with a specific antibody and characterisation of their N-glycans. The N-glycans had not been processed by enzymes localised in post-endoplasmic reticulum compartments. In contrast to MSP-1, the surface antigen SAG-1 of **Toxoplasma** gondii was efficiently transported out of the endoplasmic reticulum of insect cells and was located, at least in part, on the cell surface. No **GPI**-anchor could be detected for either of the MSP-1 constructs or SAG-1, showing that the difference in transport is a property of the individual proteins and cannot be attributed to the lack of a **GPI** -anchor. The different intracellular location and post-translational modification of recombinant proteins expressed in insect cells, as compared to the native proteins expressed in **parasites**, and the possible implications for vaccine development are discussed.

L16 ANSWER 22 OF 66 CABA COPYRIGHT 2003 CABI
AN 1999:76269 CABA
DN 990804287
TI S-Myristoylation of a glycosylphosphatidylinositol-specific phospholipase C in **Trypanosoma** brucei
AU Armah, D. A.; Mensa-Wilmot, K.
CS Department of Cellular Biology, University of Georgia, Athens, GA 30602,

USA.
SO Journal of Biological Chemistry, (1999) Vol. 274, No. 9, pp. 5931-5938. 28 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Covalent **lipid** modification of an integral membrane glycosylphosphatidylinositol-specific phospholipase C (**GPI**-PLC) from **Trypanosoma** brucei is described. Myristic acid was detected on cysteine residue(s) (ie thiomyristoylation). Thiomyristoylation occurred both co- and post-translationally. Acylated **GPI**-PLC was active against variant surface glycoprotein (VSG). The half-life of fatty acid on **GPI**-PLC was 45 minutes, indicating the dynamic nature of the modification. Deacylation in vitro decreased activity of **GPI** -PLC 18- to 30-fold. Thioacylation, from kinetic analysis, activated **GPI**-PLC by accelerating the conversion of a **GPI**-PLC.VSG complex to product. Reversible thioacylation is a novel mechanism for regulating the activity of a phospholipase C.

L16 ANSWER 23 OF 66 MEDLINE DUPLICATE 9
AN 1999375329 MEDLINE
DN 99375329 PubMed ID: 10444375
TI The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research.
AU Ferguson M A
CS Division of Molecular Parasitology and Biological Chemistry, Department of Biochemistry, The Wellcome Trust Building, University of Dundee, Dundee DD1 5EH, UK.. majferguson@bad.dundee.ac.uk
SO JOURNAL OF CELL SCIENCE, (1999 Sep) 112 (Pt 17) 2799-809. Ref: 140
Journal code: 0052457. ISSN: 0021-9533.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals; Space Life Sciences
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991229
AB The discovery of glycosylphosphatidylinositol (**GPI**) membrane anchors has had a significant impact on several areas of eukaryote cell biology. Studies of the African trypanosome, which expresses a dense surface coat of **GPI**-anchored variant surface glycoprotein, have played important roles in establishing the general structure of **GPI** membrane anchors and in delineating the pathway of **GPI** biosynthesis. The major cell-surface molecules of related **parasites** are also rich in **GPI**-anchored glycoproteins and/or **GPI**-related glycophospholipids, and differences in substrate specificity between enzymes of trypanosomal and mammalian **GPI** biosynthesis may have potential for the development of anti-**parasite** therapies. Apart from providing stable membrane anchorage, **GPI** anchors have been implicated in the sequestration of **GPI**-anchored proteins into specialised membrane microdomains, known as **lipid** rafts, and in signal transduction events.

L16 ANSWER 24 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 2000:109381 BIOSIS
DN PREV200000109381
TI Invasion by **Toxoplasma** gondii establishes a moving junction that selectively excludes host cell plasma membrane proteins on the basis of their membrane anchoring.

AU Mordue, Dana G.; Desai, Naishadh; Dustin, Michael; Sibley, L. David (1)
CS (1) Department of Molecular Microbiology, Washington University School of Medicine, 660 South Euclid Ave., Saint Louis, MO, 63110 USA
SO Journal of Experimental Medicine, (Dec. 20, 1999) Vol. 190, No. 12, pp. 1783-1792.
ISSN: 0022-1007.
DT Article
LA English
SL English
AB The protozoan **parasite Toxoplasma** gondii actively penetrates its host cell by squeezing through a moving junction that forms between the host cell plasma membrane and the **parasite**. During invasion, this junction selectively controls internalization of host cell plasma membrane components into the **parasite**-containing vacuole. Membrane **lipids** flowed past the junction, as shown by the presence of the glycosphingolipid GM1 and the cationic **lipid** label 1.1'-dihexadecyl-3-3'-3-3'-tetramethylindocarbocyanine (DiIC16). Glycosylphosphatidylinositol (**GPI**)-anchored surface proteins, such as Sca-1 and CD55, were also readily incorporated into the **parasitophorous** vacuole (PV). In contrast, host cell transmembrane proteins, including CD44, Na+/K+ ATPase, and beta1-integrin, were excluded from the vacuole. To eliminate potential differences in sorting due to the extracellular domains, **parasite** invasion was examined in host cells transfected with recombinant forms of intercellular adhesion molecule 1 (ICAM-1, CD54) that differed in their mechanism of membrane anchoring. Wild-type ICAM-1, which contains a transmembrane domain, was excluded from the PV, whereas both **GPI**-anchored ICAM-1 and a mutant of ICAM-1 missing the cytoplasmic tail (ICAM-1-Cyt-) were readily incorporated into the PV membrane. Our results demonstrate that during host cell invasion, **Toxoplasma** selectively excludes host cell transmembrane proteins at the moving junction by a mechanism that depends on their anchoring in the membrane, thereby creating a nonfusigenic compartment.

L16 ANSWER 25 OF 66 CABA COPYRIGHT 2003 CABI
AN 1999:44369 CABA
DN 990802398
TI A novel glycosylphosphatidylinositol in African trypanosomes: a possible catabolic intermediate
AU Milne, K. G.; Ferguson, M. A. J.; Englund, P. T.
CS Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee DD1 5EH, Scotland, UK.
SO Journal of Biological Chemistry, (1999) Vol. 274, No. 3, pp. 1465-1471. 41 ref.
ISSN: 0021-9258
DT Journal
LA English
AB A novel **Trypanosoma** brucei glycosylphosphatidylinositol (**GPI**), designated **lipid** X, is reported. This **GPI** is radiolabelled strongly with [3H]palmitate (and very poorly with [3H]myristate or [3H]stearate) in digitonin-permeabilized cells. The structure of **lipid** X is Man1GlcNAc-(2-O-palmitoyl)-D-myo -inositol-1-HPO4-3(lyso-palmitoylglycerol). Metabolically, **lipid** X exists as an intermediate, and can be detected only under conditions in which its formation is stimulated (eg by EDTA) or its breakdown is inhibited (eg by Co2+). **Lipid** X has not been observed previously because these conditions do not support **GPI** biosynthesis. It is speculated that **lipid** X is an intermediate in the catabolism of conventional trypanosome GPIs, possibly deriving from breakdown of glycolipid C.

L16 ANSWER 26 OF 66 CABA COPYRIGHT 2003 CABI
AN 1999:119482 CABA

DN 990806093
TI Protein S-myristoylation in Leishmania revealed with a heterologous reporter
AU Armah, D. A.; Mensa-Wilmot, K.
CS Department of Cellular Biology, University of Georgia, 724 Biological Sciences, Athens, GA 30602, USA.
SO Biochemical and Biophysical Research Communications, (1999) Vol. 256, No. 3, pp. 569-572. 15 ref.
ISSN: 0006-291X
DT Journal
LA English
AB An assay was developed to test for protein S-myristoylation in Leishmania major. A complementary DNA encoding a glycosylphosphatidylinositol-phospholipase C (**GPI**-PLC) from **Trypanosoma** cruzi, in which S-myristoylation has previously been reported, was transfected into L. major and the expressed protein tested for covalent **lipid** modifications. The results showed that **GPI**-PLC was S-myristoylated in L. major.

L16 ANSWER 27 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
AN 2000:190468 BIOSIS
DN PREV200000190468
TI Specific inhibition of an alpha-galactosyltransferase from **Trypanosoma** brucei by synthetic substrate analogues.
AU Kolb, Volker; Amann, Franz; Schmidt, Richard R.; Duszenko, Michael (1)
CS (1) Physiologisch-chemisches Institut der Universitaet Tuebingen, Hoppe-Seyler-Str. 4, D-72076, Tuebingen Germany
SO Glycoconjugate Journal, (Sept., 1999) Vol. 16, No. 9, pp. 537-544.
ISSN: 0282-0080.
DT Article
LA English
SL English
AB Since the alpha-D-galactose-(1fwdarw3)-D-galactose epitope has been identified to be the major target in the process of hyperacute rejection of xenografts transplanted from nonprimate donors to humans, specific inhibitors of alpha-galactosyltransferases are of broad interest. Using **Trypanosoma** brucei, a protozoan **parasite** causing sleeping sickness and Nagana, we have a very useful model system for the investigation of alpha-galactosyltransferase inhibitors, since the variant surface glycoprotein (VSG) accounts for about 10% of the total cell protein an this **parasite** expresses many different galactosyltransferases including the one catalysing the formation of the Galalpha1fwdarw3Gal epitope. In order to study inhibition of galactosylation on the VSG from **Trypanosoma** brucei, we designed, synthesized and tested substrate analogues of trypanosomal alpha-galactosyltransferases. Effective inhibitors were a pair of diastereoisomeric UDP-galactose analogs, in which the galactose residue is linked to UDP via a methylene bridge rather than an ester linkage. Hence, galactose cannot be transferred to the respective acceptor substrate VSG or the synthetic acceptor substrate Manalpha1fwdarw6Manalpha1S-(CH2)7-CH3, which was previously proven to replace VSG effectively (Smith et al. (1996) J Biol Chem 271:6476-82). Inhibitors have been prepared starting from 1-formyl galactal. The final condensation was performed using UMP morpholidate leading to a pair of diastereomeric compounds in 39% or 30% yield, respectively. These compounds were tested using alpha-galactosyltransferases prepared from T. brucei membranes and lactose synthetase from bovine milk. While the KM-value for UDP-galactose was determined as 59 muM on bovine lactose synthetase, the KI-values for both inhibitors were 0.3 mM and 1.1 mM respectively, showing that these inhibitors are unable to inhibit enzyme activity significantly. However, using the N-**glycan** specific alpha-galactosyltransferase from trypanosomes, the KM-value was determined as 20 muM, while the KI-values

SO Memorias do Instituto Oswaldo Cruz, (Nov., 1999) Vol. 94, No. SUPPL. 2, pp. 13.
Meeting Info.: XXVI Annual Meeting on Basic Research in Chagas' Disease and the XV Annual Meeting of Brazilian Society of Protozoology. Caxambu, Brazil November 09-11, 1999
ISSN: 0074-0276.
DT Conference
LA English
SL English

L16 ANSWER 31 OF 66 MEDLINE
AN 1998421550 MEDLINE
DN 98421550 PubMed ID: 9748303
TI The carboxyl terminus of Pneumocystis carinii glycoprotein A encodes a functional glycosylphosphatidylinositol signal sequence.
AU Guadiz G; Haidaris C G; Maine G N; Simpson-Haidaris P J
CS Departments, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, USA.
NC AI07362 (NIAID)
HL49610 (NHLBI)
HL50615 (NHLBI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Oct 2) 273 (40) 26202-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199811
ED Entered STN: 19990106
Last Updated on STN: 20030207
Entered Medline: 19981112
AB Pneumocystis carinii pneumonia is a hallmark disease associated with AIDS. An abundant glycoprotein, termed gpA, on the surface of P. carinii is considered an important factor in host-**parasite** interactions. The primary structure of ferret P. carinii gpA contains a carboxyl-terminal sequence characteristic of a signal for glycosylphosphatidylinositol (**GPI**) anchors. Here we report the capacity for this gpA carboxyl sequence to direct attachment of a secreted protein, human growth hormone (hGH), to the membranes of COS cells. A control fusion protein (hGHDAF37) was obtained which, under the direction of the **GPI** signal from decay accelerating factor, directs hGH cell surface expression. A construct (phGH2-1A30) was created similar to hGHDAF37 by fusing hGH to the putative **GPI** signal sequence encoded in the terminal 30 residues from a ferret P. carinii gpA cDNA clone. By indirect immunofluorescent staining, hGH was detected on the surface of COS cells transfected with phGH2-1A30; this surface location was confirmed by confocal laser cytometry. Metabolic labeling with [3H]ethanolamine and subsequent immunopurification of hGH from cells transfected with phGH2-1A30 confirmed that a **lipid** moiety characteristic of a conventional **GPI** anchor was linked covalently to hGH, and cell surface hGH2-1A30 fusion protein was sensitive to enzymatic cleavage by phosphatidylinositol-phospholipase C. Furthermore, hGH2-1A30 recombinant protein cofractionated with 5'-nucleotidase, a classical **GPI**-anchored membrane marker. Together, these results indicate that the carboxyl-terminal residues of ferret P. carinii gpA constitute a biologically functional **GPI** consensus domain, thus providing a potential mechanism for antigenic variation of P. carinii gpA during P. carinii pneumonia.

L16 ANSWER 32 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:232095 BIOSIS
DN PREV199800232095
TI The glycosylation of the variant surface glycoproteins and procyclic

deficiency, T. cruzi amastigotes inside mammalian cells replicated their single kinetoplast but failed at mitosis. Hence, in these protozoans, GPIs appear to be essential for nuclear division, but not for mitochondrial duplication.

L16 ANSWER 34 OF 66 MEDLINE
AN 1998062986 MEDLINE
DN 98062986 PubMed ID: 9351820
TI Identification of a species-specific inhibitor of glycosylphosphatidylinositol synthesis.
AU Sutterlin C; Horvath A; Gerold P; Schwarz R T; Wang Y; Dreyfuss M; Riezman H
CS Biozentrum of the University of Basel, Switzerland.
SO EMBO JOURNAL, (1997 Nov 3) 16 (21) 6374-83.
Journal code: 8208664. ISSN: 0261-4189.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
ED Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980108
AB Glycosylphosphatidylinositol (**GPI**)-anchoring represents a mechanism for attaching proteins to the cell surface that is used among all eukaryotes. A common core structure, EthN-P-Man3-GlcN-PI, is synthesized by sequential transfer of sugars and ethanolamine-P to PI and is highly conserved between organisms. We have screened for natural compounds that inhibit **GPI**-anchoring in yeast and have identified a terpenoid lactone, YW3548, that specifically blocks the addition of the third mannose to the intermediate structure Man2-GlcN-acyIPI. Consistent with the block in **GPI** synthesis, YW3548 prevents the incorporation of [3H]myo-inositol into proteins, transport of **GPI**-anchored proteins to the Golgi and is toxic. The compound inhibits the same step of **GPI** synthesis in mammalian cells, but has no significant activity in protozoa. These results suggest that despite the conserved core structure, the **GPI** biosynthetic machinery may be different enough between mammalian and protozoa to represent a target for anti-protozoan chemotherapy.

L16 ANSWER 35 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1997:212809 BIOSIS
DN PREV199799519313
TI Signal transduction in macrophages by glycosylphosphatidylinositols of **Plasmodium**, **Trypanosoma**, and Leishmania: Activation of protein tyrosine kinases and protein kinase C by **inositolglycan** and diacylglycerol moieties.
AU Tachado, Souvenir D. (1); Gerold, Peter; Schwarz, Ralph; Novakovic, Suzanna; McConville, Malcolm; Schofield, Louis
CS (1) Walter Eliza Hall Inst. Med. Res., VIC 3050 Australia
SO Proceedings of the National Academy of Sciences of the United States of America, (1997) Vol. 94, No. 8, pp. 4022-4027.
ISSN: 0027-8424.
DT Article
LA English
AB The perturbation of various glycosylphosphatidylinositol (**GPI**)-anchored surface proteins imparts profound regulatory signals to macrophages, lymphocytes and other cell types. The specific contribution of the **GPI** moieties to these events however is unclear. This study demonstrates that purified GPIs of **Plasmodium** falciparum, **Trypanosoma** brucei, and Leishmania mexicana origin are sufficient to initiate signal transduction when added alone to host cells as

chemically defined agonists. GPIs (10 nM-1 mu-M) induce rapid activation of the protein tyrosine kinase (PTK) p59-hck in macrophages. The minimal structural requirement for PTK activation is the evolutionarily conserved core **glycan** sequence Man-alpha-1-2Man-alpha-1-6Man-alpha-1-4GlcN1-6myo-inositol. **GPI**-associated diacylglycerols independently activate the calcium-independent epsilon isoform of protein kinase C. Both signals collaborate in regulating the downstream NF-kappa-B/rel-dependent gene expression of interleukin 1-alpha, tumor necrosis factor (TNF) alpha, and inducible NO synthase. The alkylacyl-glycerol-containing iM4 GIPL of L. mexicana, however, is unable to activate protein kinase C and inhibits TNF expression in response to other agonists, establishing signaling specificity among structurally distinct GPIs. **GPI** alone appears sufficient to mimic the activities of malaria **parasite** extracts in the signaling pathway leading to TNF expression. A mAb to **GPI** blocks TNF induction by **parasite** extracts indicating that **GPI** is a necessary agent in this response. As protozoal GPIs are closely related to their mammalian counterparts, the data indicate that GPIs do indeed constitute a novel outside-in signaling system, acting as both agonists and second messenger substrates, and imparting at least two separate signals through the structurally distinct **glycan** and fatty acid domains. These activities may underlie aspects of pathology and immune regulation in protozoal infections.

L16 ANSWER 36 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13
AN 1998:6808 BIOSIS
DN PREV199800006808
TI The surface glycoconjugates of trypanosomatid **parasites**.
AU Ferguson, Michael A. J. (1)
CS (1) Dep. Biochemistry, Univ. Dundee, Dundee DD1 4HN UK
SO Philosophical Transactions of the Royal Society of London B Biological Sciences, (Sept. 29, 1997) Vol. 352, No. 1359, pp. 1295-1302. ISSN: 0962-8436.
DT General Review
LA English
AB Insect-transmitted protozoan **parasites** of the order Kinetoplastida, suborder Trypanosomatina, include **Trypanosoma** brucei (aetiological agent of African sleeping sickness), **Trypanosoma** cruzi (aetiological agent of Chagas' disease in South and Central America) and Leishmania spp. (aetiological agents of a variety of diseases throughout the tropics and sub-tropics). The structures of the most abundant cell-surface molecules of these organisms is reviewed and correlated with the different modes of **parasitism** of the three groups of **parasites**. The major surface molecules are all glycosylphosphatidylinositol (**GPI**) -anchored glycoproteins, such as the variant surface glycoproteins of T. brucei and the surface mucins of T. cruzi, or complex glycophospholipids, such as the lipophosphoglycans and glycoinositolphospholipids of the leishmanias. Significantly, all of the aforementioned structures share a motif of Manalpha1-4GlcNalpha1-6-myo-inositol-1-HPO4-**lipid** and can therefore be considered to be members of a **GPI** superfamily.

L16 ANSWER 37 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 14
AN 97202851 EMBASE
DN 1997202851
TI Expression of a variant surface glycoprotein of **Trypanosoma** gambiense in procyclic forms of **Trypanosoma** brucei shows that the cell type dictates the nature of the glycosylphosphatidylinositol membrane anchor attached to the glycoprotein.
AU Paturiaux-Hanocq F.; Zitzmann N.; Hanocq-Quertier J.; Vanhamme L.; Rolin S.; Geuskens M.; Ferguson M.A.J.; Pays E.
CS E. Pays, Department of Molecular Biology, Free University of Brussels, 67

rue des Chevaux, B1640 Rhode Saint Genese, Belgium
SO Biochemical Journal, (1997) 324/3 (885-895).
Refs: 40
ISSN: 0264-6021 CODEN: BIJOAK
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Procyclic forms of **Trypanosoma** brucei have been genetically modified to express the major metacyclic variant surface glycoprotein (VSG variant AnTat 11.17) of **Trypanosoma** gambiense. The VSG is expressed in an intact membrane-bound form that can be detected over the entire plasma membrane, together with procyclin, and as a series of lower-molecular-mass fragments that are mostly soluble degradation products. The presence of degraded VSG in the cells and the culture medium suggests that VSG is not efficiently processed and/or efficiently folded when expressed in procyclic cells. The level of procyclin expressed on the surface of these cells is slightly reduced, although there is no difference in procyclin mRNA levels. The intact membrane-bound form of the VSG is N-glycosylated with oligomannose structures and contains a glycosylphosphatidylinositol (**GPI**) membrane anchor that can be biosynthetically labelled with [3H]ethanolamine. The anchor is sensitive to mammalian **GPI**-specific phospholipase D but, like the anchor of procyclin, it is resistant to the action of bacterial phosphatidylinositol-specific phospholipase C. This pattern of phospholipase sensitivity suggests that the **GPI** anchor acquired by VSG when expressed in procyclics is acylated on the inositol ring and therefore resembles a procyclic procyclin-type anchor rather than a trypomastigote VSG-type anchor with respect to the **lipid** structure. The VSG expressed in procyclics was sensitive to the action of a mixture of sialidase, .beta.-galactosidase and .beta.-hexosaminidase, suggesting that the VSG **GPI** anchor also contains a sialylated polylactosamine side-chain modification similar to that described for procyclin. These results indicate that the nature of the protein expressed has little influence on the post-translational modifications performed in the secretory pathway of procyclic trypanosomes.

L16 ANSWER 38 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1997:181486 BIOSIS
DN PREV199799473199
TI Molecular structure of the "low molecular weight antigen" of **Toxoplasma** gondii: A glucose alpha-1-4 N-acetylgalactosamine makes free glycosyl-phosphatidylinositols highly immunogenic.
AU Striepen, Boris; Zinecker, Christina F.; Damm, Jan B. L.; Melgers, Pedro A. T.; Gerwig, Gerrit J.; Koolen, Marck; Vliegenthart, Johannes F. G.; Dubremetz, Jean-Francois; Schwarz, Ralph T. (1)
CS (1) Zentrum Hyg. Med. Mikrobiol., Philipps-Univ. Marburg, 35037 Marburg Germany
SO Journal of Molecular Biology, (1997) Vol. 266, No. 4, pp. 797-813.
ISSN: 0022-2836.
DT Article
LA English
AB **Toxoplasma** gondii is a ubiquitous **parasitic** protozoan causing congenital infection and severe encephalitis in the course of the acquired immunodeficiency syndrome. Glycosyl-phosphatidylinositols of T. gondii have been shown to be identical with the low molecular weight antigen which elicits an early immunoglobulin M immune response in humans. A detailed study of the structures of these glycolipid antigens was performed. Radiolabelled glycolipids were extensively analysed by chemical and exoglycosidase treatments in combination with high pH anion-exchange

chromatography, gel-filtration and lectin affinity chromatography. In addition, carbohydrate fragments prepared and purified from bulk preparations of unlabelled glycolipids by high performance liquid chromatography were subjected to two-dimensional 1H nuclear magnetic resonance spectroscopy, fast-atom bombardment-mass spectrometry, and methylation linkage analysis in order to elucidate the structure of T. gondii GPIs. The following structures were identified: (ethanolamine-PO-4)Man-alpha-1-2Man-alpha-1-6(GalNAc-beta-1-4)Man-alpha-1-4GlcN-alpha-inositol-PO-4-**lipid** and the novel structure (ethanolamine-PO-4)-Man-alpha-1-2Man-alpha-1-6(Glc-alpha-1-4GalNAc-beta-14)Man-alpha-1-4GlcN-alpha-inositol-PO-4-**lipid** both with and without terminal ethanolamine phosphate. Evidence is provided, that only T. gondii GPIs bearing the unique glucose-N-acetylgalactosamine side branch are immunogenic in humans and that this structure is widely distributed among T. gondii isolates. Monoclonal antibodies have been characterized to recognize structures with different degrees of side-chain modification. We suggest that these reagents in combination with recently devised techniques for insertional mutagenisis in T. gondii should greatly facilitate the cloning of genes essential for **GPI** side-chain modification.

L16 ANSWER 39 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16
AN 1997:455209 BIOSIS
DN PREV199799754412
TI The trans-sialidase of **Trypanosoma** cruzi is anchored by two different **lipids**.
AU Augusti, Rosalia; Couto, Alicia S.; Campetella, Oscar E.; Frasch, Alberto C. C.; De Lederkremer, Rosa M. (1)
CS (1) CIHIDECAR, Dep. Quimica Organica, Fac. Ciencias Exactas Naturales, Univ. de Buenos Aires, Pabellon 2, Ciudad Universitaria, Buenos Aires Argentina
SO Glycobiology, (1997) Vol. 7, No. 6, pp. 731-735.
ISSN: 0959-6658.
DT Article
LA English
AB The trans-sialidase from the trypomastigote stage of **Trypanosoma** cruzi was metabolically labeled with (3H)-palmitic acid and purified by immunoprecipitation h a monoclonal antibody. The action of PI-PLC on the immunoprecipitate released a **lipid** that was analyzed by TLC. Lyso-1-O-hexadecylglycerol and N-palmitoyl-sphinganine were obtained in a 1:3 ratio. A comparison with the **GPI** anchors present in the different stages of T. cruzi was made.

L16 ANSWER 40 OF 66 LIFESCI COPYRIGHT 2003 CSA
AN 97:89961 LIFESCI
TI Glycosyl phosphatidylinositol myristoylation in African trypanosomes
AU Werbovetz, K.A.; Englund, P.T.*
CS Dep. Biol. Chem., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, USA
SO MOL. BIOCHEM. PARASITOL., (1997) vol. 85, no. 1, pp. 1-7.
ISSN: 0166-6851.
DT Journal
TC General Review
FS K
LA English
SL English
AB The variant surface glycoprotein (VSG) of **Trypanosoma** brucei plays a vital role in the survival of this **parasite** within its host. In a process of antigenic variation the **parasite** switches expression from one VSG to another, and thereby evades the host's immune response. The VSG forms a coat covering the entire surface of the **parasite**, with roughly 10 super(7) VSG molecules tightly packed in a monolayer. Each VSG molecule is attached to the bloodstream-form T.

brucei cell surface by a glycosyl phosphatidylinositol (**GPI**) anchor. **GPI** anchors are also used for cell surface tethering of proteins by other eukaryotes, and all protein-linked **GPI** anchors have the same **glycan** core structure: Ethanolamine-p-6-Man alpha 1-2-Man alpha 1-6-Man alpha 1-4-GlcN. This core structure is linked at its reducing end to either a phosphatidylinositol or an inositol-phospho-ceramide. The T. brucei VSG **GPI** is unique, however, in that both fatty acids in the diacylglycerol portion of its phosphatidylinositol are myristate. The specificity for myristoylation is high, as the initial chemical analysis showed no hint of any other fatty acid besides myristate. It is intriguing that the trypanosome chooses to incorporate myristate into its **GPI** anchor because this **parasite** cannot synthesize fatty acids de novo and must therefore import its entire supply from the host. Myristate is a relatively rare fatty acid in the blood of mammals, comprising only about 1% of the total fatty acyl residues. In addition, there is no detectable beta -oxidation of the more abundant longer chain fatty acids such as palmitate and stearate, so it is unlikely that trypanosomes obtain significant quantities of myristate by chain shortening. The total myristate concentration (including free fatty acid, phospholipid, triglyceride, and cholesterol ester) is only about 27 mu M in rat serum.

L16 ANSWER 41 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 17

AN 1996:381642 BIOSIS

DN PREV199699103998

TI Identification of complete precursors for the glycosylphosphatidylinositol protein anchors of **Trypanosoma** cruzi.

AU Heise, Norton; Raper, Jayne; Buxbaum, Laurence U.; Peranovich, Tereza M. S.; Cardoso De Almeida, Maria Lucia (1)

CS (1) Dep. Biol. Mol., Lab. Parasitol. Mol., ULB, Rue des Chevaux 67, B-1640 Rhode-St-Genese Belgium

SO Journal of Biological Chemistry, (1996) Vol. 271, No. 28, pp. 16877-16887. ISSN: 0021-9258.

DT Article

LA English

AB The survival of **Trypanosoma** cruzi, the causative agent of Chagas' disease, depends vitally on proteins and glycoconjugates that mediate the **parasite**/host interaction. Since most of these molecules are attached to the membrane by glycosylphosphatidylinositol (**GPI**), alternative means of chemotherapeutic intervention might emerge from **GPI** biosynthesis studies. The structure of the major 1G7 antigen **GPI** has been fully characterized by us (Guther, M. L. S., Cardoso de Almeida, M. L., Yoshida, N., and Ferguson, M. A. J. (1992) J. Biol. Chem. 267,6820-6828; Heise, N., Cardoso de Almeida, M. L., and Ferguson, M. A. J. (1995) Mol. Biochem. **Parasitol**. 70, 71-84), and based on its properties we now report the complete precursor glycolipids predicted to be transferred to the nascent protein. Migrating closely to **Trypanosoma** brucei glycolipid A on TLC, such species, named glycolipids A-like 1 and A-like 2, were labeled with tritiated palmitic acid, myo-inositol, glucosamine, and mannose, but surprisingly only the less polar glycolipid A-like 1 incorporated ethanolamine. The predicted products following nitrous acid deamination and digestion with phospholipases A2, C, and D confirmed their **GPI** nature. Evidence that they may represent the anchor transferred to the 1G7 antigen came from the following analyses: (i) alpha-mannosidase treatments indicated that only one mannose was amenable to removal; (ii) their **lipid** moiety was identified as sn-1-alkyl-2-acylglycerol due to their sensitivity to phospholipase A-2 (PLA-2), mild base and by direct high performance TLC analysis of the corresponding benzoylated diradylglycerol components; and (iii) both glycolipids incorporated 3H-fatty acid only in the sn-2- and not in the sn-1-alkyl position as previously found in the **GPI** of the mature 1G7 antigen. Based on the differential

(3H)ethanolamine incorporation pattern and the recent report that an aminoethylphosphonic acid (AEP) replaces ethanolamine phosphate (EtNH-2-PO-4) in the **GPI** in epimastigote sialoglycoproteins (Previato, J. O., Jones, C., Xavier, M. T., Wait, R., Travassos, L. R., Parodi, A. J., and Mendonca-Previato, L. (1995) J. Biol. Chem. 270, 7241-7250) it is proposed that glycolipid A-like 2 contains AEP and A-like 1 EtNH-2-PO-4. In the in vitro cell-free system both glycolipids were synthesized simultaneously and do not seem to bear a precursor/product relationship. Among the various components synthesized in vitro a glycolipid C-like corresponding to a form of glycolipid A-like 1 acylated on the inositol was also characterized. Phenylmethylsulfonyl fluoride, an inhibitor known to block the addition of ethanolamine phosphate in T. brucei but not in mammalian cells, also inhibits the synthesis of glycolipids A-like and C-like in T. cruzi, indicating that the putative trypanosome EtNH-2-PO-4/AEP transferase(s) might represent a potential target for chemotherapy.

L16 ANSWER 42 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 18
AN 1996:472205 BIOSIS
DN PREV199699201761
TI **Lipid** metabolism in **Trypanosoma** brucei: Utilization of myristate and myristoyllysophosphatidylcholine for myristoylation of glycosyl phosphatidylinositols.
AU Werbovetz, Karl A. (1); Englund, Paul T.
CS (1) Dep. Biol. Chem., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 USA
SO Biochemical Journal, (1996) Vol. 318, No. 2, pp. 575-581. ISSN: 0264-6021.
DT Article
LA English
AB Myristate is the exclusive fatty acid species in the glycosyl phosphatidylinositol (**GPI**) anchor of the **Trypanosoma** brucei variant surface glycoprotein (VSG). (3H)Myristate can be incorporated into T. brucei GPIs by two distinct processes known as fatty acid remodelling and myristate exchange. Myristoyllysophosphatidylcholine (M-LPC) can also serve as a myristate donor for VSG in trypanosomes (Bowes, Samad, Jiang, Weaver and Mellors (1993) J. Biol. Chem. 268, 13885-13892). We have studied in detail the myristoylation of GPIs using a (3H)M-LPC substrate. Labelling of VSG and free GPIs by (3H)M-LPC in cultured trypanosomes occurred at the same rate as with (3H)myristate. Concurrent with **GPI** labelling, there was rapid hydrolysis of (3H)M-LPC to generate extracellular (3H)myristate. Experiments in a trypanosomal cell-free system indicated that **GPI** labelling by fatty acid remodelling and myristate exchange was also equally efficient with (3H)M-LPC and (3H)myristate. Furthermore, both ATP and CoA are required for the myristoylation of GPIs by (3H)M-LPC. These experiments suggest that **GPI** myristoylation from M-LPC involves hydrolysis of M-LPC to free myristate. To address the physiological importance of myristate and M-LPC in VSG myristoylation, we radiolabelled trypanosomes in vivo with both substrates in medium containing serum, and found that (3H)myristate labelled VSG and GPIs more efficiently. Thus, VSG myristoylation by free myristate may be favoured in bloodstream trypanosome infections.

L16 ANSWER 43 OF 66 CABA COPYRIGHT 2003 CABI
AN 96:136775 CABA
DN 960805415
TI Ceramide 1-phosphate is released from a glycoinositolphosphoceramide of **Trypanosoma** cruzi by rat blood plasma
AU Lederkremer, R. M. de; Lima, C.; Vila, M. del C.; De Lederkremer, R. M.
CS Departamento de Quimica Organica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina.

SO Molecular and Biochemical Parasitology, (1996) Vol. 79, No. 2, pp. 219-223. 23 ref.
ISSN: 0166-6851
DT Journal
LA English
AB [3H]Palmitic acid-labelled LPPG (lipopeptidophosphoglycan from the surface of **Trypanosoma** cruzi epimastigotes) was incubated with rat blood plasma (a source of **GPI**-PLD (glycosylphosphatidylinositol-phospholipase D)). Radioactive products were dissolved first with ether and then with CHCl3:CH30H:12 M HCl (10:10:0.1, v/v). No radioactivity was extracted with ether, but the radioactive products were dissolved in the latter solvent. Analysis by TLC revealed a ceramide phosphate. This product chromatographed similarly to a standard of ceramide phosphate obtained from sphingomyelin. A purified commercial sample of **GPI** -PLD gave the same result as the plasma, confirming that the specificity of the enzyme is defined by the phosphoinositol-**glycan** and not the **lipid** moiety.

L16 ANSWER 44 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 19
AN 1995:256718 BIOSIS
DN PREV199598271018
TI Structural characterization of the major glycosylphosphatidylinositol membrane-anchored glycoprotein from epimastigote forms of **Trypanosoma** cruzi Y-strain.
AU Previato, Jose O.; Jones, Christopher; Xavier, Marcia T.; Wait, Robin; Travassos, Luiz R.; Parodi, Armando J.; Mendonca-Previato, Lucia (1)
CS (1) Dep. Microbiol. Geral, Univ. Federal do Rio de Janeiro, Rio de Janeiro 21944970 Brazil
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 13, pp. 7241-7250.
ISSN: 0021-9258.
DT Article
LA English
AB We have investigated the structure of the glycosylphosphatidylinositol (**GPI**) anchor and the O-linked **glycan** chains of the 40/45-kDa glycoprotein from the cell surface of the protozoan **parasite Trypanosoma** cruzi. This glycoconjugate is the major acceptor for sialic acid transferred by trans-sialidase of T. cruzi Y-strain, epimastigote form. The **GPI** anchor was liberated by treatment with hot alkali, and the phosphoinositol-oligosaccharide moiety was characterized. Unusually the glucosamine was 6-O-substituted with 2-aminoethylphosphonate, and 2-aminoethylphosphonate was also present on the third mannose residue distal to glucosamine, partially replacing the ethanolamine phosphate. The beta-eliminated reduced oligosaccharide chains showed that two novel classes of O-linked N-acetylglucosamine oligosaccharide were present. The first series had the structures Galp-beta-1-3GlcNAc-ol; Galp-beta-1-6(Galp-beta-1-3)GlcNAc-ol; and Galp-beta-1-2Galp-beta-1-6(Galp-beta-1-3)GlcNAc-ol, whereas the other series had a 1-4 linkage to N-acetylglucosaminitol and had structures Galp-beta-1-4GlcNAc-ol, Galp-beta-1-6(Galp-beta-1-4)GlcNAcol, and Galp-beta-1-2Galp-beta-1-6(Galp-beta-1-4)GlcNAc-ol. We have also investigated the kinetics of in vitro sialylation of these O-linked oligosaccharides by the T. cruzi transsialidase and have shown that incorporation of one molecule of sialic acid hinders entry of a second molecule when two potential acceptor sites are present.

L16 ANSWER 45 OF 66 CABA COPYRIGHT 2003 CABI
AN 95:192796 CABA
DN 950808503
TI **Glycan** requirements of glycosylphosphatidylinositol phospholipase C from **Trypanosoma** brucei
AU Morris, J. C.; Lei, PingSheng; Shen, TsungYing; Mensa-Wilmot, K.; Lei, P. S.; Shen, T. Y.

CS Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA.
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 6, pp. 2517-2524. 36 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Glycosylphosphatidylinositol phospholipase C (**GPI**-PLC) from **Trypanosoma** brucei and phosphatidylinositol phospholipase C (PI-PLC) from Bacillus sp. both cleave glycosylphosphatidylinositols (GPIs). However, phosphatidylinositol, which is efficiently cleaved by PI-PLC, is a very poor substrate for **GPI**-PLC. **GPI**-PLC substrate requirements were examined using glycoinositol analogs of **GPI** components as potential inhibitors. Glucosaminyl(alpha 1 right arrow 6)-D-myo-inositol (GlcN(alpha 1 right arrow 6)Ins), GlcN-(alpha 1 right arrow 6)Ins 1,2-cyclic phosphate, GlcN(alpha 1 right arrow 6)Ins, GlcN-(alpha 1 right arrow 6)Ins 1,2-cyclic phosphate, GlcN(alpha 1 right arrow 6)-2-deoxy-Ins, and GlcN(alpha 1 right arrow 6)Ins 1-dodecyl phosphonate inhibited **GPI**-PLC. GlcN(alpha 1 right arrow 6)Ins was as effective as Man-(alpha 1 right arrow 4)GlcN(alpha 1 right arrow 6)Ins; it was surmised that GlcN(alpha 1 right arrow 6)Ins is the crucial **glycan** motif for **GPI**-PLC recognition. Inhibition by GlcN(alpha 1 right arrow 6)Ins 1,2-cyclic phosphate suggests product inhibition since GPIs cleaved by **GPI**-PLC possess a GlcN(alpha 1 right arrow 6)Ins, 1,2-cyclic phosphate at the terminus of the residual **glycan**. The effectiveness of GlcN-(alpha 1 right arrow 6)-2-deoxyl-Ins indicates that the D-myo-inositol (Ins) 2-hydroxyl is not required for substrate recognition, although it is probably essential for catalysis. GlcN(alpha 1 right arrow 6)-2-deoxy-L-myo-inositol, unlike GlcN(alpha 1 right arrow 6)-2-deoxy-Ins, had no effect on **GPI**-PLC; hence, **GPI** -PLC can distinguish between the two enantiomers of Ins. Surprisingly, GlcN(alpha 1 right arrow 6)Ins 1,2-cyclic phosphate was not a potent inhibitor of Bacillus cereus PI-PLC, and GlcN(alpha 1 right arrow 6)Ins had no effect on the enzyme. However, both GlcN(alpha 1 right arrow 6)Ins 1-phosphate and GlcN(alpha 1 right arrow 6)Ins 1-dodecyl phosphonate were competitive inhibitors of PI-PLC. These observations suggest an important role for a phosphoryl group at the Ins 1-position in PI-PLC recognition of GPIs. Other studies indicate that abstraction of a proton from the Ins 2-hydroxyl is not an early event in PI-PLC cleavage of GPIs. Furthermore, both GlcN(alpha 1 right arrow 6)-2-deoxy-Ins 1-phosphate and GlcN(alpha 1 right arrow 6)-2-deoxy-L-myo-inositol inhibited PI-PLC without affecting **GPI**-PLC. Last, the aminoglycoside G418 stimulated PI-PLC, but had no effect on **GPI**-PLC. Thus, these enzymes represent mechanistic subclasses of **GPI** phospholipases C, distinguishable by their sensitivity to GlcN(alpha 1 right arrow 6)Ins derivatives and aminoglycosides. Possible allosteric regulation of PI-PLC by GlcN(alpha 1 right arrow 6)Ins analogs is discussed.

L16 ANSWER 46 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 95138327 EMBASE
DN 1995138327
TI Yeast Gaa1p is required for attachment of a completed **GPI** anchor onto proteins.
AU Hamburger D.; Egerton M.; Riezman H.
CS Biozentrum of University of Basel, Klingelbergstrasse 70,CH-4056 Basel, Switzerland
SO Journal of Cell Biology, (1995) 129/3 (629-639).
ISSN: 0021-9525 CODEN: JCLBA3
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English

SL English
AB Anchoring of proteins to membranes by glycosylphosphatidylinositols (GPIs) is ubiquitous among all eukaryotes and heavily used by **parasitic** protozoa. **GPI** is synthesized and transferred en bloc to form **GPI**-anchored proteins. The key enzyme in this process is a putative **GPI**:protein transamidase that would cleave a peptide bond near the COOH terminus of the protein and attach the **GPI** by an amide linkage. We have identified a gene, GAA1, encoding an essential ER protein required for **GPI** anchoring. gaa1 mutant cells synthesize the complete **GPI** anchor precursor at nonpermissive temperatures, but do not attach it to proteins. Overexpression of GAA1 improves the ability of cells to attach anchors to a **GPI** -anchored protein with a mutant anchor attachment site. Therefore, Gaalp is required for a terminal step of **GPI** anchor attachment and could be part of the putative **GPI**:protein transamidase.

L16 ANSWER 47 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 96016080 EMBASE
DN 1996016080
TI Glycosyl inositolphospholipid-anchored structures in Herpetomonas davidi.
AU Butikofer P.; Boschung M.
CS Inst. of Biochem./Molecular Biology, University of Bern, Buhlstrasse 28,3012 Bern, Switzerland
SO Molecular and Biochemical Parasitology, (1995) 74/1 (65-75). ISSN: 0166-6851 CODEN: MBIPDP
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Glycosyl inositolphospholipid (**GPI**)-anchored structures in the monogenetic **parasite** Herpetomonas davidi, were labeled with [3H]myristic acid and [3H]glucosamine, and characterized by enzymatic and chemical treatments that are typical for the identification of **GPI** anchors. [3H]Myristate incorporated into two different pools of **GPI**-linked structures that could be separated by chromatography on octyl-Sepharose. One pool consisted of three **GPI**-anchored proteins with apparent molecular masses of 21, 31 and 45 kDa, and the **GPI lipid** moieties were identified as alkyl-lysoglycerols. The label in the other pool associated with lipopeptidophosphoglycan (LPPG)-like structures of approximately 12-kDa molecular mass, containing ceramide-type **GPI lipid** anchors. While protein **GPI** anchors could also be labeled using [3H]glucosamine as radiolabeled **GPI** anchor precursor, hardly any radioactivity was incorporated into the LPPG-like structures. H. davidi is one of the few organisms identified to date that synthesizes two structurally different **lipid** moieties for **GPI** anchoring of membrane components.

L16 ANSWER 48 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 94231557 EMBASE
DN 1994231557
TI The effects of phenylmethylsulfonyl fluoride on inositol-acylation and fatty acid remodeling in African trypanosomes.
AU Guther M.L.S.; Masterson W.J.; Ferguson M.A.J.
CS Department of Biochemistry, University of Dundee,Dundee DD1 4HN, United Kingdom
SO Journal of Biological Chemistry, (1994) 269/28 (18694-18701). ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 004 Microbiology
030 Pharmacology

037 Drug Literature Index
LA English
SL English
AB Phenylmethylsulfonyl fluoride (PMSF) has been shown to inhibit the addition of ethanolamine phosphate to glycosylphosphatidylinositol (**GPI**) intermediates in **Trypanosoma** brucei (Masterson W. J., and Ferguson, M. A. J. (1991) EMBO J. 10, 2041-2045). Here we show that the Man3-GlcN-PI intermediate that accumulates in the presence of PMSF can undergo fatty acid remodeling, suggesting that the fatty acid remodeling enzymes are not specific for ethanolamine phosphate-containing **GPI** intermediates. We also show that PMSF inhibits the acylation of the inositol residue of **GPI** intermediates in bloodstream form T. brucei. Pulse-chase experiments demonstrate that glycolipid C (ethanolamine-PO4-Man3-GlcN-(acyl)PI) is not an obligatory precursor of glycolipid A (ethanolamine-PO4-Man3-GlcN-PI) and that glycolipid C can be converted to glycolipid A. These data suggest a model where glycolipid C is the terminal product of the **GPI** biosynthetic pathway, in dynamic equilibrium with glycolipid A. The inhibition of ethanolamine phosphate addition and inositol acylation by PMSF was also observed for procyclic forms of T. brucei but not for mammalian HeLa cells. These results suggest differences between the relevant **parasite** and mammalian enzymes.

L16 ANSWER 49 OF 66 CABA COPYRIGHT 2003 CABI
AN 95:5903 CABA
DN 940806453
TI Free and protein-linked glycoinositolphospholipids in **Trypanosoma** cruzi
AU Lederkremer, R. M. de; De Lederkremer, R. M.; : Cardoso-de-Almeida, M. L. [EDITOR]; Mendonca-Previato, L. [EDITOR]; Ramalho-Pinto, F. J. [EDITOR]; Silva, A. M. da [EDITOR]; Travassos, L. R. [EDITOR]
CS Departamento de Quimica Organica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellon II, Ciudad Universitaria 1428 Buenos Aires, Argentina.
SO Brazilian Journal of Medical and Biological Research, (1994) Vol. 27, No. 2, pp. 239-242. 15 ref.
Meeting Info.: Proceedings of the "GPI Membrane Anchors Structure and Function 1994" Meeting, Angra dos Reis, RJ, Brazil, 20-26 February 1994..
ISSN: 0100-879X
DT Conference Article; Journal
LA English
AB Two glycoinositolphospholipids (GIPL A and GIPL B) were purified from epimastigotes of **Trypanosoma** cruzi at the logarithmic phase of growth (2 days). [3H]-palmitic acid was incorporated into 1-O-hexadecyl-2-O-palmitoylglycerolin GIPL A and into a sphinganine ceramide with palmitic acid and lignoceric acid as the fatty acids in GIPL B. The **lipids** could be released by incubation with phosphatidylinositol-specific phospholipase C (PI-PLC) or glycosylphosphatidylinositol phospholipase D (**GPI**-PLD) from rat serum. Microheterogeneity was demonstrated, as well as substitution by galactose, which is mainly in the furanose configuration as was previously described for the LPPG. However, methylation analysis indicated that 20% of the galactose is present as terminal pyranose units. In infective trypomastigotes, [3H]-palmitic acid was incorporated into the anchor of the Tc-85 glycoprotein. The **lipid** cleaved by phospholipase C digestion was identified as 1-O-hexadecylglycerol and the main oligosaccharide has the structure of the conserved core of all **GPI** anchors. [3H]-palmitic acid-labelled Tc-85 released into the culture medium as membrane vesicles showed 80% resistance to the action of PI-PLC. However, after mild alkaline hydrolysis, part of the radioactivity was released by the enzyme.

L16 ANSWER 50 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

20
AN 1994:301217 BIOSIS
DN PREV199497314217
TI Structural study on the glycosyl-phosphatidylinositol anchor and the asparagine-linked sugar chain of a soluble form of CD59 in human urine.
AU Nakano, Yasuko (1); Noda, Keiichi; Endo, Tamao; Kobata, Akira; Tomita, Motowo
CS (1) Dep. Physiol. Chem., Sch. Pharm. Sci., Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142 Japan
SO Archives of Biochemistry and Biophysics, (1994) Vol. 311, No. 1, pp. 117-126.
ISSN: 0003-9861.
DT Article
LA English
AB CD59 is an 18-kDa glycoprotein widely expressed on human cells. An important structural feature of CD59 is its attachment to the cell surface via a glycosyl-phosphatidylinositol (**GPI**) anchor. CD59, like many **GPI**-anchored proteins, has been found in urine, serum, and other body fluids. The structures of the **GPI** anchor and the asparagine-linked sugar chain of a soluble form of CD59 in urine, U-CD59, were determined. Purified U-CD59 released 1 mol of inositol per mole of protein by nitrous acid deamination, which cleaved between glucosamine and inositol present commonly in the **GPI** anchor. This indicates that a **GPI** anchor, which ended with inositol, is linked at the carboxy terminus of U-CD59. The peptide containing an asparagine-linked sugar chain and the peptide containing a **glycan** portion of the **GPI** anchor were isolated after trypsin digestion of U-CD59. The asparagine-linked sugar chains and the **glycan** portion of the **GPI** anchor were isolated from these peptides following hydrazinolysis or deamination and dephosphorylation, respectively. Their structures were analyzed by sequential exoglycosidase digestion and methylation analyses. The structures of the asparagine-linked sugar chains of U-CD59 were biantennary complex type, only 4.2% of which are monosialylated. The backbone structure of the **GPI** anchor was similar to that of **Trypanosoma** brucei variant surface glycoprotein, but showed significant variations in its side-chain moieties. This is the first detailed structural analysis of the human **GPI** anchor and the first detailed analysis of the carboxylterminal structure of the soluble-form **GPI**-anchored protein. The results indicate that the backbone structure of the **GPI** anchor is conserved from **parasites** to human and that at least a part of the soluble-form **GPI**-anchored protein has the structure produced by the action of **glycan**-phosphatidylinositol-specific phospholipase D.

L16 ANSWER 51 OF 66 MEDLINE DUPLICATE 21
AN 93252779 MEDLINE
DN 93252779 PubMed ID: 8486622
TI Trypanosome metabolism of myristate, the fatty acid required for the variant surface glycoprotein membrane anchor.
AU Doering T L; Pessin M S; Hoff E F; Hart G W; Raben D M; Englund P T
CS Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
NC AI21334 (NIAID)
HL39086 (NHLBI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 May 5) 268 (13) 9215-22.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199306
ED Entered STN: 19930618

Last Updated on STN: 19980206
Entered Medline: 19930604

AB The trypanosome variant surface glycoprotein (VSG) is anchored to the outer leaflet of the **parasite** plasma membrane by a glycosyl phosphatidylinositol (**GPI**). The VSG anchor is unique among GPIs in containing exclusively dimyristoylglycerol as its **lipid** moiety. Myristate is incorporated into the anchor precursor by sequential deacylation and specific reacylation with myristate. Although myristate is required for the VSG anchor, trypanosomes cannot synthesize this fatty acid and must import their entire supply from the host bloodstream, where it exists in low abundance. Chemical analysis of these **parasites** reveals that most of their myristate is in VSG protein, with no major **lipid** storage form. Unexpectedly, when these cells are radiolabeled with [3H]myristate in culture, most of the label is incorporated into phospholipids, with little into VSG. This apparent contradiction is explained by the fact that trypanosomes in culture medium elongate much of the [3H]myristate into palmitate and stearate, probably because the medium (with only 5% serum) contains limiting amounts of these fatty acids. In contrast, trypanosomes radiolabeled in whole blood (with higher concentrations of palmitate and stearate) do not modify most of the [3H]myristate, and instead utilize the major portion of it for **GPI** synthesis. Our studies suggest that bloodstream trypanosomes have evolved highly efficient means of directing myristate into the **GPI** biosynthetic pathway.

L16 ANSWER 52 OF 66 CABA COPYRIGHT 2003 CABI
AN 95:18900 CABA
DN 940807089
TI The carbohydrate structures of **Trypanosoma** brucei brucei MITat 1.6 variant surface glycoprotein. A re-investigation of the C-terminal **glycan**
AU Strang, A. M.; Allen, A. K.; Holder, A. A.; Halbeek, H. van; Van Halbeek, H.
CS Complex Carbohydrate Research Center and Department of Biochemistry, University of Georgia, 220 Riverbend Road, Athens, GA 30602-4712, USA.
SO Biochemical and Biophysical Research Communications, (1993) Vol. 196, No. 3, pp. 1430-1439. 26 ref.
ISSN: 0006-291X
DT Journal
LA English
AB The glycosylphosphatidylinositol (**GPI**) carbohydrate structure of soluble variant surface glycoprotein (sVSG) of **Trypanosoma** brucei (strain 427, clone 121, MITat 1.6) was investigated. The sVSG was prepared by osmotic lysis of trypanosome cells in the presence of Zn2+. Glycopeptides generated by Pronase digestion of the sVSG were purified by gel permeation and ion-exchange chromatography, and structurally characterized by 1H and 31P nuclear magnetic resonance spectroscopy in combination with chemical composition analyses. 2 glycopeptide fractions were obtained which were homogeneous in their peptide and heterogeneous in their carbohydrate structures. The fraction representing the N-glycosylation site of the VSG contained high-mannose type oligosaccharides with structures Man7-9GlcNAc2 linked to Asn-Ala-Thr. The other fraction contained the membrane-anchoring C-terminal **glycan** of the VSG attached to Asp and its oligosaccharide structures were of the **GPI** type.

L16 ANSWER 53 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 22
AN 1994:14382 BIOSIS
DN PREV199497027382
TI Structural analysis of glycosyl-phosphatidylinositol membrane anchor of the **Toxoplasma** gondii tachyzoite surface glycoprotein gp23.
AU Tomavo, Stanislas (1); Dubremetz, Jean-Francois; Schwarz, Ralph T.

CS (1) Dep. Microbiol. Immunol., Stanford Univ. Sch. Med., Fairchild Build. D-305, Stanford, CA 94305-5402 USA
SO Biology of the Cell (Paris), (1993) Vol. 78, No. 3, pp. 155-162. ISSN: 0248-4900.
DT Article
LA English
AB In this study we describe the biochemical features of the **Toxoplasma** gondii tachyzoite surface glycoprotein, gp23, demonstrating that it is attached to the **parasite** membrane by a glycosyl-phosphatidyl inositol anchor. Gp23 was metabolically labeled with tritiated palmitate, myristate, ethanolamine, inositol, glucosamine, mannose and galactose, as expected for a **GPI**-anchor structure. Gp23 was released from the surface of living **parasites** after treatment with phosphatidylinositol-specific phospholipase C (PI-PLC) and the resulting water-soluble protein was immunoprecipitated with a monoclonal antibody specific for gp23. The **GPI**-core **glycan** was generated after aqueous-HF dephosphorylation followed by nitrous acid deamination and its carbohydrate structure was analyzed using selective exo- and endoglycosidase treatments. Finally, the phosphatidylinositol moiety of gp23 was characterized using PI-PLC and phospholipase A-2 (PLA-2) digestions. Our cumulative data suggest that gp23 of T gondii tachyzoites contains a modified **GPI**-backbone similar to the mammalian Thy-1 anchor, consisting of a conserved core structure (ethanolamine-PO-4-6-Man-alpha-1-2-Man-alpha-1-6-Man-alpha-1-4-GlcN-alpha-1-6-PI) bearing beta-linked N-acetylgalactosamine residue(s).

L16 ANSWER 54 OF 66 CABA COPYRIGHT 2003 CABI
AN 93:102892 CABA
DN 930806702
TI Structural characterization of a novel class of glycophosphosphingolipids from the protozoan Leptomonas samueli
AU Previato, J. O.; Mendonca-Previato, L.; Jones, C.; Wait, R.; Fournet, B.
CS Departamento de Microbiologia Geral, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 34, pp. 24279-24286. 44 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Aqueous phenol extraction of Leptomonas samueli released into the aqueous layer a chloroform/methanol/water-soluble glycophosphosphingolipid fraction. Alkaline degradation and purification by gel filtration chromatography resulted in a tetrasaccharide (phosphatidylinositol (PI)-oligosaccharide A), and a pentasaccharide (PI-oligosaccharide B), each containing 2 mol of 2-aminoethylphosphonate and one mol of phosphate. A structure for PI-oligosaccharide A and PI-oligosaccharide B is suggested. The glycophosphosphingolipids of Leptomonas have features in common with the glycolipids of both Leishmania and **Trypanosoma** cruzi, resembling the former by the alpha (1 right arrow 3) linkage of mannose to the **GPI** core, while the 2-aminoethylphosphonate substituent on O-6 of glucosamine and the presence of ceramide in place of glycerol **lipids** is more reminiscent of T. cruzi. These data are thought to lend some support to the hypothesis that both T. cruzi and Leishmania evolved from a Leptomonas-like ancestor.

L16 ANSWER 55 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 23
AN 1993:5327 BIOSIS
DN PREV199395005327
TI Biosynthesis of glycolipid precursors for glycosylphosphatidylinositol membrane anchors in a **Toxoplasma** gondii cell-free system.
AU Tomavo, Stanislas (1); Dubremetz, Jean-Francois; Schwarz, Ralph T.
CS (1) Dep. Microbiology Immunology, Stanford University School Medicine,

Fairchild Bldg. D-305, Stanford, Calif. 94305-5402
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 30, pp. 21446-21458. ISSN: 0021-9258.
DT Article
LA English
AB Toxoplasmosis, a disease that affects humans and a wide variety of mammals is caused by **Toxoplasma** gondii, the obligate intracellular coccidian protozoan **parasite**. Most T. gondii research has focused on the rapidly growing invasive form, the tachyzoite, which expresses five major surface proteins attached to the **parasite** membrane by glycosylphosphatidylinositol (**GPI**) anchors. We have recently reported the purification and partial characterization of candidate precursor glycolipids (GPIs) from metabolically labeled **parasites** and have presented evidence that these GPIs have a linear **glycan** backbone sequence indistinguishable from the **GPI** core **glycan** of the major tachyzoite surface protein, P30. In this report, we describe a cell-free system derived from tachyzoite membranes which is capable of catalyzing **GPI** biosynthesis. Incubation of the membrane preparations with radioactive sugar nucleotides (GDP-(3H)mannose or UDP-(3H)GlcNAc) resulted in incorporation of radiolabel into numerous glycolipids. By using a combinationa of chemical/enzymatic tests and chromatographic analysis, a series of incompletely glycosylated **lipid** species and mature GPIs have been identified. We have also established the involvement of Dol-P-mannose in the synthesis of T. gondii GPIs by demonstrating that the incorporation of (3H)mannose into the mannosylated GPIs is stimulated by dolichylphosphate and inhibited by amphomycin. In addition, increasing the concentration of nonradioactive GDP mannose resulted in a loss of radiolabeled from the first easily detectable **GPI** precursor, GlcN-PI, and a concomitant appearance of the radioactivity into mannosylated glycolipids. Altogether, our data suggest that the **GPI** core **glycan** in T. gondii is assembled via sequential glycosylation of phosphatidylinositol, was proposed for the biosynthesis of GPIs in **Trypanosoma** brucei. In contrast to T. brucei, preliminary experiments indicate that the core **glycan** of some GPIs synthesized by the T. gondii cell-free system is modified by N-acetylgalactosamine similar to the situation for mammalian Thy-1.

L16 ANSWER 56 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:144260 CABA
DN 920801094
TI An inositol phosphate **glycan** derived from a **Trypanosoma** brucei glycosyl-phosphatidylinositol mimics some of the metabolic actions of insulin
AU Misek, D. E.; Saltiel, A. R.
CS Department of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA.
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 3, pp. 16266-16273. 73 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Some of the acute actions of insulin may be mediated by an enzyme-modulating inositol phosphate **glycan**, produced by the insulin-sensitive hydrolysis of glycosyl-phosphatidylinositol (**GPI**) that is structurally similar to a membrane protein anchor. An inositol **glycan** fragment from the structurally characterized T. brucei variant surface glycoprotein **GPI** anchor is evaluated for insulin-mimetic antilipolytic activity. The fragment specifically and dose-dependently inhibits isoproterenol-stimulated lipolysis. Like the effect of insulin, **glycan**-induced antilipolysis is blocked by the low Km cAMP phosphodiesterase inhibitor imazodan (CI-914) and the serine/threonine phosphatase inhibitor, okadaic acid, suggesting that the

activation of both cAMP phosphodiesterase and serine/threonine protein phosphatases are necessary. Moreover, this fragment causes a specific and dose-dependent inhibition of both microsomal glucose-6-phosphatase (EC 3.1.3.9) and cytosolic fructose-1,6-bisphophatase (EC 3.1.3.11) activity. Additionally, direct addition of the **glycan** to hepatocytes caused marked inhibition of glucose production from pyruvate. These results suggest that the direct modification of the activities of these 2 gluconeogenic enzymes by an inositol **glycan** may play a role in the inhibition of glucose output by insulin and provide the first evidence for the insulin-mimetic properties of a chemically characterized inositol **glycan**.

L16 ANSWER 57 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:100939 CABA
DN 920879896
TI A family of glycolipids from **Toxoplasma** gondii. Identification of candidate glycolipid precursor(s) for **Toxoplasma** gondii glycosylphosphatidylinositol membrane anchors
AU Tomavo, S.; Dubremetz, J. F.; Schwarz, R. T.
CS Department of Microbiology and Immunology, Stanford University, School of Medicine, Fairchild Building, D305, Stanford, CA 94305-5402, USA.
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 17, pp. 11721-11728. 34 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Four major glycolipids were extracted from T. gondii tachyzoites which were metabolically labelled with tritiated glucosamine, mannose, palmitic And myristic acid, ethanolamine, and inositol. These glycolipids were found to share the following properties with the glycolipid moiety of the glycosylphosphatidylinositol anchor (**GPI** anchor) of the major surface protein, P30, of T. gondii: a nonacetylated glucosamine-inositol phosphate linkage; sensitivity toward phosphatidylinositol-specific phospholipase C and nitrous acid; identity of HF-dephosphorylated **GPI glycan** backbone between 3 glycolipids and the HF-dephosphorylated core **glycan** of the **GPI** anchor of the major surface protein P30; the presence of a linear core **glycan** structure blocked by an ethanolamine phosphate residue(s). It is concluded that, taken together with the nature of radiolabelled precursors incorporated into these glycolipids, the data indicate that these GPIs are involved in the biosynthesis of the **GPI**-membrane anchors of T. gondii.

L16 ANSWER 58 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 24
AN 1992:258683 BIOSIS
DN BA93:135008
TI STRUCTURAL STUDIES ON THE GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR OF **TRYPANOSOMA**-CRUZI 1G7-ANTIGEN THE STRUCTURE OF THE **GLYCAN** CORE.
AU GUTHER M L S; DE ALMEIDA M L C; YOSHIDA N; FERGUSON M A J
CS DEP. BIOCHEMISTRY, UNIVERSITY DUNDEE, DUNDEE DD14HN, SCOTL.
SO J BIOL CHEM, (1992) 267 (10), 6820-6828.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB The 1G7-antigen is expressed by the infective metacyclic trypomastigote stage of the protozoan **parasite Trypanosoma** cruzi. The 1G7-antigen is a 90-kDa glycoprotein, present at about 40,000 copies/cell, which is anchored in the plasma membrane via a glycosylphosphatidylinositol (**GPI**) membrane anchor. The **glycan** of the **GPI** anchor has been isolated from immunopurified 1G7-antigen and its structure determined using a

combination of methylation linkage analysis and exoglycosidase sequencing. The structure of the **glycan** is Man.alpha.1-2Man.alpha.1-2Man.alpha.1-6Man.alpha.1-4GlcNH2. The glucosamine residue is in glycosidic linkage to a phosphatidylinositol moiety. The penultimate nonreducing .alpha.-Man residue is substituted with phosphate, which is most likely part of an ethanolamine phosphate bridge linking the **GPI** anchor to the 1G7-antigen polypeptide. The **glycan** sequence was obtained from 1.1 nmol of glycoprotein isolated from a detergent lysate of whole cells. The procedures reported here represent a high sensitivity protocol for determining **GPI glycan** structures from small quantities of biological material. The structure of the 1G7-antigen **GPI** anchor is consistent with the conserved core structure of all **GPI** anchors analyzed to date and is similar to that of the T. cruzi lipopeptidophosphoglycan. The biosynthesis of **GPI** anchors and lipopeptidophosphoglycan in T. cruzi is discussed in the light of this structural homology.

L16 ANSWER 59 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:100639 CABA
DN 920800045
TI Developmental variation of glycosylphosphatidylinositol membrane anchors in **Trypanosoma** brucei
AU Field, M. C.; Menon, A. K.; Cross, G. A. M.
CS Laboratory of Molecular Parasitology, 1230 York Ave., The Rockefeller University, New York, NY 10021, USA.
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 8, pp. 5324-5329. 30 ref.
ISSN: 0021-9258
DT Journal
LA English
AB Using a membrane preparation from procyclic trypanosomes, which is capable of synthesizing glycosylphosphatidyl-inositol (**GPI**) **lipids** upon the addition of nucleotide sugars, it was found that inositol type, and the mature ethanolamine-phosphate-containing precursors are exclusively acylated. It is suggested that the differences between the bloodstream and procyclic form **GPI** biosynthetic intermediates can be accounted for by the developmental regulation of an inositol acylhydrolase, which is active only in the bloodstream form, and a glyceride fatty acid remodeling system, which is only partially functional in the procyclic form.

L16 ANSWER 60 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:76440 CABA
DN 920878449
TI Galactose-containing glycosylphosphatidylinositols in **Trypanosoma** brucei
AU Mayor, S.; Menon, A. K.; Cross, G. A. M.
CS Room 15-420, Department of Pathology, College of Physicians and Surgeons of Columbia University, 630 W. 168th St., New York, NY 10032, USA.
SO Journal of Biological Chemistry, (1992) Vol. 267, No. 2, pp. 754-761. 43 ref.
ISSN: 0021-9258
DT Journal
LA English
AB The purification and partial characterization of candidate precursor glycolipids (P2 and P3) from T. brucei is reported. P2 and P3 contain ethanolamine-phosphate-Man alpha 1-2Man alpha 1-6Man alpha 1-GlcN linked glycosidically to an inositol residue, as do all the **GPI** anchors that have been structurally characterized. The anchors on mature VSGs contain a heterogenously branched galactose structure attached alpha 1-3 to the mannose residue adjacent to the glucosamine. The identification of free GPIs that appear to be similarly galactosylated is also reported. These glycolipids contain diacylglycerol and alpha -galactosidase-

sensitive **glycan** structures which are indistinguishable from the glycans derived from galactosylated VSG **GPI** anchors. The relevance of these galactosylated GPIs to the biosynthesis of VSG **GPI** anchors is discussed.

L16 ANSWER 61 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:144237 CABA
DN 920800759
TI A glycosylphosphatidylinositol protein anchor from procyclic stage **Trypanosoma** brucei: **lipid** structure and biosynthesis
AU Field, M. C.; Menon, A. K.; Cross, G. A. M.
CS Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.
SO EMBO Journal, (1991) Vol. 10, No. 10, pp. 2731-2739. 49 ref.
ISSN: 0261-4189
DT Journal
LA English
AB It was shown, by metabolic labelling with [3H]fatty acids, that the procyclic acidic repetitive protein (PARP) anchor of the procyclic stage of T. brucei contains palmitate esterified to inositol, and stearate at sn-1, in a monoacylglycerol moiety. Using pulse-chase labelling, it was shown that both fatty acids are incorporated into the **GPI** anchor from a large pool of metabolic precursors, rather than directly from acyl-CoA. The addition of the **GPI** anchor moiety to PARP was dependent on de novo protein synthesis, and this is thought to exclude the possibility that incorporation of fatty acids into PARP can occur by a remodelling of pre-existing **GPI** anchors. The phosphatidylinositol (PI) species that are utilized for **GPI** biosynthesis were shown to be a subpopulation of the cellular PI molecular species. It is concluded that these observations may be of general validity since several other eukaryotic membrane proteins (e.g human erythrocyte acetylcholine esterase and decay accelerating factor) have been reported to contain palmitoylated inositol residues.

L16 ANSWER 62 OF 66 MEDLINE
AN 92379441 MEDLINE
DN 92379441 PubMed ID: 1823620
TI Glycosylation in **parasitic** protozoa of the trypanosomatidae family.
AU Zamze S
CS Department of Biochemistry, University of Oxford, UK.
SO GLYCOCONJUGATE JOURNAL, (1991 Dec) 8 (6) 443-7. Ref: 46
Journal code: 8603310. ISSN: 0282-0080.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199209
ED Entered STN: 19921018
Last Updated on STN: 19921018
Entered Medline: 19920925
AB Over the last few years enormous interest has been shown in the structures of the **glycan** moieties of various **parasite** surface glycoconjugates. Structures have been determined for the glyco-components of glycosylphosphatidylinositol (**GPI**) protein membrane anchors, for asparagine-linked oligosaccharides, and for the glycans of complex glycolipids. The following attempts to illustrate a few of the most salient observations with regard to the structures and possible functions of **parasite** surface glycans.

L16 ANSWER 63 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

25
AN 1991:5395 BIOSIS
DN BA91:5395
TI STRUCTURE OF THE GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR OF THE LEISHMANIA-MAJOR PROMASTIGOTE SURFACE PROTEASE.
AU SCHNEIDER P; FERGUSON M A J; MCCONVILLE M J; MEHLERT A; HOMANS S W; BORDIER C
CS INSTITUT DE BIOCHIMIE, UNIVERSITE DE LAUSANNE, CH-1066 EPALINGES, SWITZERLAND.
SO J BIOL CHEM, (1990) 265 (28), 16955-16964.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB In common with many other plasma membrane glycoproteins of eukaryotic origin, the promastigote surface protease (PSP) of the protozoan **parasite** Leishmania contains a glycosyl-phosphatidylinositol (**GPI**) membrane anchor. The **GPI** anchor of Leishmania major PSP was purified following proteolysis of the PSP and analyzed by two-dimensional 1H-1H NMR, compositional and methylation linkage analyses, chemical and enzymatic modifications, and amino acid sequencing. From these results, the structure of the **GPI**-containing peptide was found to be Asp-Gly-Gly-Asn-ethanolamine-PO4-6Man.alpha.1-2Man.alpha.1-6Man.alpha.1-4GlcN.alpha.1- 6myo-inositol-1-PO4-(1-alkyl-2-acyl-glycerol). The **glycan** structure is identical to the conserved **glycan** core regions of the **GPI** anchor of **Trypanosoma** brucei variant surface glycoprotein and rat brain Thy-1 antigen; supporting the notion that this portion of GPIs are highly conserved. The phosphatidylinosital moiety of the PSP anchor is unusual, containing a fully saturated, unbranched 1-O-alkyl chain (mainly C24:0) and a mixture of fully saturated unbranched 2-O-acyl chains (C12:0, C14:0, C16:0, and C18:0). This **lipid** composition differs significantly from those of the GPIs of T. brucei variant surface glycoprotein and mammalian erythrocyte acetylcholinesterase but is similar to that of a family of glycosylated phosphoinositides found uniquely in Leishmania.

L16 ANSWER 64 OF 66 CABA COPYRIGHT 2003 CABI
AN 92:76490 CABA
DN 920878804
TI Glycolipid precursors for the membrane anchor of **Trypanosoma** brucei variant surface glycoproteins
AU Mayor, S.; Menon, A. K.; Cross, G. A. M.
CS The Rockefeller University, 1230 York Ave., New York, NY 10021, USA.
SO Journal of Biological Chemistry, (1990) Vol. 265, No. 11, pp. 6164-6173. 52 ref.
ISSN: 0021-9258
DT Journal
LA English
AB The purification and partial characterization of a candidate precursor glycolipid (P2) and of a compositionally similar glycolipid (P3) from T. brucei is reported. The primary structure of the **glycan** portions of P2 and P3 were analyzed by a combination of selective chemical fragmentation and enzymatic **glycan** sequencing at the subnanomolar level. The glycans were generated by deamination, NaB3H4 reduction, and dephosphorylation of glycolipids purified from different trypanosome variants. **Glycan** fragments derived from biosynthetically labelled glycolipids were also analyzed. The cumulative data strongly suggest that P2 and P3 contain ethanolamine-phosphate-Man alpha 1-2Man alpha 1-6Man alpha 1-GlcN linked glycosidically to an inositol residue, as do all the **GPI** anchors that have been structurally characterized. The structural similarities suggest that **GPI** membrane anchors are derived from common precursor glycolipids that become variably modified during or after addition to newly synthesized proteins.

L16 ANSWER 65 OF 66 CABA COPYRIGHT 2003 CABI
AN 91:3555 CABA
DN 910868855
TI A novel pathway for **glycan** assembly: biosynthesis of the glycosyl-phosphatidylinositol anchor of the trypanosome variant surface glycoprotein
AU Masterson, W. J.; Doering, T. L.; Hart, G. W.; Englund, P. T.
CS Department of Biological Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.
SO Cell (Cambridge), (1989) Vol. 56, No. 5, pp. 793-800. 29 ref.
ISSN: 0092-8674
DT Journal
LA English
AB The trypanosome variant surface glycoprotein (VSG), is anchored to the plasma membrane by a glycosyl-phosphatidylinositol (**GPI**) moiety. This glycolipid is assembled first as a precursor (glycolipid A) that is then covalently attached to the newly synthesized polypeptide. A trypanosome cell-free system, capable of performing all of the steps in the biosynthesis of the **glycan** portion of glycolipid A, was developed. Using [3H]sugar nucleotides as substrates, several biosynthetic intermediates were identified. From structural analyses of these intermediates, a pathway for **GPI** biosynthesis is proposed. Based on comparisons between the VSG **GPI** anchor and similar structures in other cells, it is believed that this same pathway will apply to the **GPI** anchors, and the related insulin-mediator compound, of higher eukaryotes.

L16 ANSWER 66 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 26
AN 1988:222733 BIOSIS
DN BA85:111968
TI DEVELOPMENTALLY REGULATED PHOSPHOLIPASE C-MEDIATED RELEASE OF THE MAJOR SURFACE GLYCOPROTEIN OF AMASTIGOTES OF **TRYPANOSOMA**-CRUZI.
AU ANDREWS N W; ROBBINS E S; LEY V; HONG K S; NUSSENZWEIG V
CS DEP. BACTERIOL., OSAKA UNIV. SCH. MED., INST. MICROBIAL DISEASES, OSAKA 565, JPN.
SO J EXP MED, (1988) 167 (2), 300-314.
CODEN: JEMEAV. ISSN: 0022-1007.
FS BA; OLD
LA English
AB The surface of amastigotes of **Trypanosoma** cruzi is covered by Ssp-4, a major stage-specific glycoprotein. Ssp-4 is anchored to the cell membrane by **GPI**. It can be metabolically labeled with [3H]myristic acid, and is converted into a hydrophilic form by treatment with the **glycan**-specific phospholipase C of T. brucei, or after lysis of the **parasites** in non-ionic detergents. The hydrophilic form of Ssp-4 is recognized by antibodies to the cross-reactive determinant of the variant surface glycoprotein of African trypanosomes. Ssp-4 is progressively shed during the intra- or extracellular development of amastigotes preceding their transformation into epi- and trypomastigotes. We show here that T. cruzi contains a phospholipase C and that most shed Ssp-4 is hydrophilic, does not contain myristic acid, and reacts with anti-CRD. These observations provide strong evidence that phospholipase C mediates the release of this glycosyl-phosphatidylinositol-anchored protein under physiological conditions, as the **parasite** ' undergoes differentiation.

WEST Search History

DATE: Tuesday, May 06, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L26	L24 and glycan	13	L26
L25	L24 and lipidic	3	L25
L24	L23 and malaria	239	L24
L23	L22 and vaccine	263	L23
L22	L21 and (lipid$ or glycan)	279	L22
L21	gpi and plasmodium	308	L21
L20	L19 and malaria$	72	L20
L19	L18 and vaccin$	337	L19
L18	L17 and gpi	441	L18
L17	L16 and (gpi or glycan)	885	L17
L16	((424/$)!.CCLS.)	81012	L16
L15	L14 and (parasit$ or plasmodium)	177	L15
L14	L13 and vaccin$	624	L14
L13	L12 and (glycan or lipid$)	681	L13
L12	l10 and gpi	734	L12
L11	L10 and gpi adj10 glycan	0	L11
L10	l7 or l8 or L9	13214	L10
L9	((514/8)!.CCLS.)	1862	L9
L8	((424/184.1)!.CCLS.)	1692	L8
L7	((530/350)!.CCLS.)	10439	L7
L6	schofield-l.in.	2	L6
L5	L4 and vaccin?	1	L5
L4	L3 and inositolglycan	11	L4
L3	gpi	3246	L3
L2	schofield-louis.in.	0	L2
L1	schofield-louis.in.	0	L1

END OF SEARCH HISTORY

WEST

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Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: WO 200024406 A1 EP 1126857 A1 AU 200011425 A

L6: Entry 1 of 2 File: DWPI May 4, 2000

DERWENT-ACC-NO: 2000-350579
DERWENT-WEEK: 200150
COPYRIGHT 2003 DERWENT INFORMATION LTD
TITLE: Activation of helper T cells using glycosylphosphatidylinositol for treatment and/or prophylaxis of a mammalian disease condition caused by a microorganism infection e.g. Leishmaniasis

INVENTOR: HANSEN, D; SCHOFIELD, L

PRIORITY-DATA: 1998AU-0006758 (October 27, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200024406 A1	May 4, 2000	E	116	A61K031/72
EP 1126857 A1	August 29, 2001	E	000	A61K031/72
AU 200011425 A	May 15, 2000		000	A61K031/72

INT-CL (IPC): A61 K 31/72; A61 K 31/73; A61 K 39/008; A61 K 39/015; A61 K 121:00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 2. Document ID: WO 200015254 A1 EP 1113815 A1 AU 9958420 A

L6: Entry 2 of 2 File: DWPI Mar 23, 2000

DERWENT-ACC-NO: 2000-271259
DERWENT-WEEK: 200140
COPYRIGHT 2003 DERWENT INFORMATION LTD
TITLE: Inducing immune response against a microorganism, useful particularly for treating or preventing malaria, by administering an inositolglycan domain of glycosylphosphatidylinositol

INVENTOR: SCHOFIELD, L

PRIORITY-DATA: 1998AU-0005893 (September 14, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200015254 A1	March 23, 2000	E	100	A61K039/015
EP 1113815 A1	July 11, 2001	E	000	A61K039/015
AU 9958420 A	April 3, 2000		000	A61K039/015

INT-CL (IPC): A61 K 39/002; A61 K 39/015

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Term	Documents
SCHOFIELD-L.DWPI,EPAB,JPAB,USPT,PGPB.	3
SCHOFIELD-LS	0
SCHOFIELD-L.IN..USPT,PGPB,JPAB,EPAB,DWPI,TDBD.	2
(SCHOFIELD-L.IN.).USPT,PGPB,JPAB,EPAB,DWPI,TDBD.	2

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WEST

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Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: WO 200024406 A1 EP 1126857 A1 AU 200011425 A

L6: Entry 1 of 2 File: DWPI May 4, 2000

DERWENT-ACC-NO: 2000-350579
DERWENT-WEEK: 200150
COPYRIGHT 2003 DERWENT INFORMATION LTD
TITLE: Activation of helper T cells using glycosylphosphatidylinositol for treatment and/or prophylaxis of a mammalian disease condition caused by a microorganism infection e.g. Leishmaniasis

INVENTOR: HANSEN, D; SCHOFIELD, L

PRIORITY-DATA: 1998AU-0006758 (October 27, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200024406 A1	May 4, 2000	E	116	A61K031/72
EP 1126857 A1	August 29, 2001	E	000	A61K031/72
AU 200011425 A	May 15, 2000		000	A61K031/72

INT-CL (IPC): A61 K 31/72; A61 K 31/73; A61 K 39/008; A61 K 39/015; A61 K 121:00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: WO 200015254 A1 EP 1113815 A1 AU 9958420 A

L6: Entry 2 of 2 File: DWPI Mar 23, 2000

DERWENT-ACC-NO: 2000-271259
DERWENT-WEEK: 200140
COPYRIGHT 2003 DERWENT INFORMATION LTD
TITLE: Inducing immune response against a microorganism, useful particularly for treating or preventing malaria, by administering an inositolglycan domain of glycosylphosphatidylinositol

INVENTOR: SCHOFIELD, L

PRIORITY-DATA: 1998AU-0005893 (September 14, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200015254 A1	March 23, 2000	E	100	A61K039/015
EP 1113815 A1	July 11, 2001	E	000	A61K039/015
AU 9958420 A	April 3, 2000		000	A61K039/015

INT-CL (IPC): A61 K 39/002; A61 K 39/015

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

Generate Collection | Print

Term	Documents
SCHOFIELD-L.DWPI,EPAB,JPAB,USPT,PGPB.	3
SCHOFIELD-LS	0
SCHOFIELD-L.IN..USPT,PGPB,JPAB,EPAB,DWPI,TDBD.	2
(SCHOFIELD-L.IN.).USPT,PGPB,JPAB,EPAB,DWPI,TDBD.	2

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E10          1     SCHOFIELD M N/AU
E11          1     SCHOFIELD M R/AU
E12          1     SCHOFIELD M S/AU

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L1           9 "SCHOFIELD LORRAINE"/AU

=> s e3
L2          41 "SCHOFIELD LOUIS"/AU

=> s l2 and gpi
L3          26 L2 AND GPI

=> s l3 and vaccin?
L4           7 L3 AND VACCIN?

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5           4 DUP REM L4 (3 DUPLICATES REMOVED)

=> d bib ab 1-4

L5   ANSWER 1 OF 4  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN   2002:471725  BIOSIS
DN   PREV200200471725
TI   Synthetic GPI as a candidate antitoxic vaccine in a
     model of malaria.
AU   Schofield, Louis (1); Hewitt, Michael C.; Evans, Krystal;
     Siomos, Mary-Anne; Seeberger, Peter H.
CS   (1) Walter and Eliza Hall Institute of Medical Research, Royal Melbourne
     Hospital, Post Office, Melbourne, VIC, 3050: schofield@wehi.edu.au,
     seeberg@mit.edu Australia
SO   Nature (London), (15 August, 2002) Vol. 418, No. 6899, pp. 785-789.
     http://www.nature.com/nature/. print.
     ISSN: 0028-0836.
DT   Article; Letter
LA   English
AB   The malaria parasite Plasmodium falciparum infects 5-10% of the world's
     population and kills two million people annually. Fatalities are thought
     to result in part from pathological reactions initiated by a malarial
     toxin. Glycosylphosphatidylinositol (GPI) originating from the
     parasite has the properties predicted of a toxin; however, a requirement
     for toxins in general and GPI in particular in malarial
     pathogenesis and fatality remains unproven. As anti-toxic vaccines
     can be highly effective public health tools, we sought to determine
     whether anti-GPI vaccination could prevent pathology
     and fatalities in the Plasmodium berghei/rodent model of severe malaria.
     The P. falciparum GPI glycan of the sequence
     NH2-CH2-CH2-PO4-(Manalpha1-2)6Manalpha1-2Manalpha1-6Manalpha-1-
     4GlcNH2alpha1-6myo-inositol-1,2-cyclic-phosphate was chemically
     synthesized, conjugated to carriers, and used to immunize mice. Recipients
     were substantially protected against malarial acidosis, pulmonary oedema,
     cerebral syndrome and fatality. Anti-GPI antibodies neutralized
     pro-inflammatory activity by P. falciparum in vitro. Thus, we show that
     GPI is a significant pro-inflammatory endotoxin of parasitic
     origin, and that several disease parameters in malarious mice are
     toxin-dependent. GPI may contribute to pathogenesis and
     fatalities in humans. Synthetic GPI is therefore a prototype
     carbohydrate anti-toxic vaccine against malaria.

L5   ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2003 ACS
AN   2000:290843  CAPLUS
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PROCESSING COMPLETED FOR L3
L6 14 DUP REM L3 (12 DUPLICATES REMOVED)

=> d bib ab 1-14

L6 ANSWER 1 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2002:452549 BIOSIS
DN PREV200200452549
TI Genes for glycosylphosphatidylinositol toxin biosynthesis in Plasmodium falciparum.
AU Delorenzi, Mauro; Sexton, Adrienne; Shams-Eldin, Hosam; Schwarz, Ralph T.; Speed, Terry; **Schofield, Louis (1)**
CS (1) The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, 3050: schofield@wehi.edu.au Australia
SO Infection and Immunity, (August, 2002) Vol. 70, No. 8, pp. 4510-4522. print.
ISSN: 0019-9567.
DT Article
LA English
AB About 2.5 million people die of Plasmodium falciparum malaria every year. Fatalities are associated with systemic and organ-specific inflammation initiated by a parasite toxin. Recent studies show that glycosylphosphatidylinositol (**GPI**) functions as the dominant parasite toxin in the context of infection. GPIs also serve as membrane anchors for several of the most important surface antigens of parasite invasive stages. **GPI** anchoring is a complex posttranslational modification produced through the coordinated action of a multi-component biosynthetic pathway. Here we present eight new genes of P. falciparum selected for encoding homologs of proteins essential for **GPI** synthesis: PIG-A, PIG-B, PIG-M, PIG-O, GPI1, GPI8, GAA-1, and DPM1. We describe the experimentally verified mRNA and predicted amino acid sequences and in situ localization of the gene products to the parasite endoplasmic reticulum. Moreover, we show preliminary evidence for the PIG-L and PIG-C genes. The biosynthetic pathway of the malaria parasite **GPI** offers potential targets for drug development and may be useful for studying parasite cell biology and the molecular basis for the pathophysiology of parasitic diseases.

L6 ANSWER 2 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2002:471725 BIOSIS
DN PREV200200471725
TI Synthetic **GPI** as a candidate antitoxic vaccine in a model of malaria.
AU **Schofield, Louis (1)**; Hewitt, Michael C.; Evans, Krystal; Siomos, Mary-Anne; Seeberger, Peter H.
CS (1) Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Post Office, Melbourne, VIC, 3050: schofield@wehi.edu.au, seeberg@mit.edu Australia
SO Nature (London), (15 August, 2002) Vol. 418, No. 6899, pp. 785-789. http://www.nature.com/nature/. print.
ISSN: 0028-0836.
DT Article; Letter
LA English
AB The malaria parasite Plasmodium falciparum infects 5-10% of the world's population and kills two million people annually. Fatalities are thought to result in part from pathological reactions initiated by a malarial toxin. Glycosylphosphatidylinositol (**GPI**) originating from the parasite has the properties predicted of a toxin; however, a requirement for toxins in general and **GPI** in particular in malarial pathogenesis and fatality remains unproven. As anti-toxic vaccines can be highly effective public health tools, we sought to determine whether anti-

Hospital, Parkville, VIC, 3050 Australia
SO Parasite Immunology (Oxford), (Dec., 1999) Vol. 21, No. 12, pp. 609-617. ISSN: 0141-9838.
DT Article
LA English
SL English
AB Glycosylphosphatidylinositols (GPIs) and related glycoconjugates of parasite origin have been shown to regulate both the innate and acquired immune systems of the host. This is achieved through the activation of novel **GPI**-dependent signalling pathways in macrophages, lymphocytes and other cell types. Parasite GPIs impart at least two distinct signals to host cells through the structurally distinct inositolphosphoglycan (IPG) and fatty acid domains. Binding of IPG to as yet uncharacterized cell surface receptor(s) leads to activation of src-family protein tyrosine kinases: depending upon structure, **GPI** -derived fatty acids can either activate or antagonize protein kinase C, and may enter the sphingomyelinase pathway. The degree of fatty acid saturation may also contribute to signalling activity. Thus, variation in structure of parasite GPIs imparts different properties of signal transduction upon this class of glycolipid. The divergent activities of GPIs from various protozoal taxa reflect global aspects of the host/parasite relationship, suggesting that **GPI** signalling is a central determinant of disease in malaria, leishmaniasis and both American and African trypanosomiases.

L6 ANSWER 7 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 1999:87812 BIOSIS
DN PREV199900087812
TI CD1d-restricted immunoglobulin G formation to **GPI**-anchored antigens mediated by NKT cells.
AU **Schofield, Louis (1)**; McConville, Malcolm J.; Hansen, Diana; Campbell, A. Stewart; Fraser-Reid, Bert; Grusby, Michael J.; Tachado, Souvenir D.
CS (1) Walter and Eliza Hall Inst. Med. Res., Post Office, R. Melbourne Hosp., Victoria 3050 Australia
SO Science (Washington D C), (Jan. 8, 1999) Vol. 283, No. 5399, pp. 225-229. ISSN: 0036-8075..
DT Article
LA English
AB Immunoglobulin G (IgG) responses require major histocompatibility complex (MHC)-restricted recognition of peptide fragments by conventional CD4+ helper T cells. Immunoglobulin G responses to glycosylphosphatidylinositol (**GPI**)-anchored protein antigens, however, were found to be regulated in part through CD1d-restricted recognition of the **GPI** moiety by thymus-dependent, interleukin-4-producing CD4+, natural killer cell antigen 1.1 ((NK1.1)+) helper T cells. The CD1-NKT cell pathway regulated immunogobulin G responses to the **GPI**-anchored surface antigens of Plasmodium and Trypanosoma and may be a general mechanism for rapid, MHC-unrestricted antibody responses to diverse pathogens.

L6 ANSWER 8 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 1997:212809 BIOSIS
DN PREV199799519313
TI Signal transduction in macrophages by glycosylphosphatidylinositols of Plasmodium, Trypanosoma, and Leishmania: Activation of protein tyrosine kinases and protein kinase C by inositolglycan and diacylglycerol moieties.
AU Tachado, Souvenir D. (1); Gerold, Peter; Schwarz, Ralph; Novakovic, Suzanna; McConville, Malcolm; **Schofield, Louis**
CS (1) Walter Eliza Hall Inst. Med. Res., VIC 3050 Australia
SO Proceedings of the National Academy of Sciences of the United States of

America, (1997) Vol. 94, No. 8, pp. 4022-4027.
ISSN: 0027-8424.
DT Article
LA English
AB The perturbation of various glycosylphosphatidylinositol (**GPI**)-anchored surface proteins imparts profound regulatory signals to macrophages, lymphocytes and other cell types. The specific contribution of the **GPI** moieties to these events however is unclear. This study demonstrates that purified GPIs of Plasmodium falciparum, Trypanosoma brucei, and Leishmania mexicana origin are sufficient to initiate signal transduction when added alone to host cells as chemically defined agonists. GPIs (10 nM-1 mu-M) induce rapid activation of the protein tyrosine kinase (PTK) p59-hck in macrophages. The minimal structural requirement for PTK activation is the evolutionarily conserved core glycan sequence Man-alpha-1-2Man-alpha-1-6Man-alpha-1-4GlcN1-6myo-inositol. **GPI**-associated diacylglycerols independently activate the calcium-independent epsilon isoform of protein kinase C. Both signals collaborate in regulating the downstream NF-kappa-B/rel-dependent gene expression of interleukin 1-alpha, tumor necrosis factor (TNF) alpha, and inducible NO synthase. The alkylacyl-glycerol-containing iM4 GIPL of L. mexicana, however, is unable to activate protein kinase C and inhibits TNF expression in response to other agonists, establishing signaling specificity among structurally distinct GPIs. **GPI** alone appears sufficient to mimic the activities of malaria parasite extracts in the signaling pathway leading to TNF expression. A mAb to **GPI** blocks TNF induction by parasite extracts indicating that **GPI** is a necessary agent in this response. As protozoal GPIs are closely related to their mammalian counterparts, the data indicate that GPIs do indeed constitute a novel outside-in signaling system, acting as both agonists and second messenger substrates, and imparting at least two separate signals through the structurally distinct glycan and fatty acid domains. These activities may underlie aspects of pathology and immune regulation in protozoal infections.

L6 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 1997:431499 CAPLUS
DN 127:158835
TI Glycosyl-phosphatidylinositols in protozoa structure, biosynthesis and intracellular localization
AU Zinecker, Christina; Gerold, Peter; Azzouz, Nahid; Striepen, Boris; Schmidt, Almut; Berhe, Saba; Kimmel, Jurgen; Keddes, Mamdouh H.; Blackman, Michael J.; **Schofield, Louis**; Ogun, Sola; Damm, Jan B. L.; Melgers, Pedro A. T.; Koolen, Marck; Gerwig, Gerrit J.; Vliegenhardt, Johannes F. G.; Dubremetz, Jean F.; Holder, Anthony A.; Eckert, Volker; Capdeville, Yvonne; Tachado, Souvenir D.; Schwarz, Ralph T.
CS Med. Zentrum fur Hygiene und Med. Mikrobiologie, Philipps-Universitat Marburg, Germany
SO Indian Journal of Biochemistry & Biophysics (1997), 34(1&2), 105-109
CODEN: IJBBBQ; ISSN: 0301-1208
PB National Institute of Science Communication
DT Journal
LA English
AB We are investigating the structure and biosynthesis of glycosyl-phosphatidylinositols (**GPI**) in the protozoa Toxoplasma gondii, Plasmodium falciparum, Plasmodium yoelii and Paramecium primaurelia. This comparison of structural and biosynthesis data should lead us to common and individual features of the **GPI**-biosynthesis and transport in different organisms.

L6 ANSWER 10 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1996:160129 BIOSIS
DN PREV199698732264

TI Glycosylphosphatidylinositol toxin of Plasmodium induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway.

AU Tachado, Souvenir D. (1); Gerold, Peter; McConville, Malcolm J.; Baldwin, Tracey; Quilici, Denis; Schwarz, Ralph T.; **Schofield, Louis**

CS (1) Immunoparasitol. Unit, Walter and Eliza Hall Inst. Med. Res., Post Office, Royal Melbourne Hosp., Parkville, VIC 3050 Australia

SO Journal of Immunology, (1996) Vol. 156, No. 5, pp. 1897-1907. ISSN: 0022-1767.

DT Article

LA English

AB In this study, we demonstrate that glycosylphosphatidylinositol (**GPI**) is a major toxin of Plasmodium falciparum origin responsible for nitric oxide (NO) production in host cells. Purified malarial **GPI** is sufficient to induce NO release in a time- and dose-dependent manner in macrophages and vascular endothelial cells, and regulates inducible NO synthase expression in macrophages. **GPI** -induced NO production was blocked by the NO synthase-specific inhibitor L-N-monomethylarginine. **GPI** also synergizes with IFN-gamma in regulating NO production. The structurally related molecules dipalmitoylphosphatidylinositol and iM4 glycoinositolphospholipid from Leishmania mexicana had no such activity, and the latter antagonized IFN-gamma-induced NO output. **GPI** activates macrophages by initiating an early onset tyrosine kinase-mediated signaling process, similar to that induced by total parasite extracts. The tyrosine kinase antagonists tyrphostin and genistein inhibited the release of NO by parasite extracts and by **GPI**, alone or in combination with IFN-gamma, demonstrating the involvement of one or more tyrosine kinases in the signaling cascade. **GPI**-induced NO release was also blocked by the protein kinase C inhibitor calphostin C, demonstrating a role for protein kinase C in **GPI**-mediated cell signaling, and by pyrrolidine dithiocarbamate, indicating the involvement of the NF-kappa-B/c-rel family of transcription factors in cell activation. A neutralizing mAb to malarial **GPI** inhibited NO production induced by **GPI** and total malarial parasite extracts in human vascular endothelial cells and murine macrophages, indicating that **GPI** is a necessary agent of parasite origin in parasite-induced NO output. Thus, in contrast to dipalmitoylphosphatidylinositol and glycoinositolphospholipids of Leishmania, malarial **GPI** initiates a protein tyrosine kinase- and protein kinase C-mediated signal transduction pathway, regulating inducible NO synthase expression with the participation of NF-kappa-B-rel, which leads to macrophage and vascular endothelial cell activation and downstream production of NO. These events may play a role in the etiology of severe malaria.

L6 ANSWER 11 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7

AN 1996:160128 BIOSIS

DN PREV199698732263

TI Glycosylphosphatidylinositol toxin of Plasmodium up-regulates intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin expression in vascular endothelial cells and increases leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction.

AU **Schofield, Louis (1)**; Novakovic, Susanna; Gerold, Peter; Schwarz, Ralph T.; McConville, Malcolm J.; Tachado, Souvenir D.

CS (1) Immunoparasitol. Unit, Walter and Eliza Hall Inst. Med. Res., Post Office, Royal Melbourne Hosp., VIC 3050 Australia

SO Journal of Immunology, (1996) Vol. 156, No. 5, pp. 1886-1896. ISSN: 0022-1767.

DT Article

LA English

MISSING OPERATOR LASMODIUM)_
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l7 and (parasit? or plasmodium)
L8 1890 L7 AND (PARASIT? OR PLASMODIUM)

=> s l8 and trypanosoma or leishmaina or toxoplasma or candida or fungal)
UNMATCHED RIGHT PARENTHESIS 'FUNGAL)'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s l8 and (trypanosoma or leishmaina or toxoplasma or candida or fungal)
L9 767 L8 AND (TRYPANOSOMA OR LEISHMAINA OR TOXOPLASMA OR CANDIDA OR
FUNGAL)

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 281 DUP REM L9 (486 DUPLICATES REMOVED)

=> s. l10 and vaccin?
L11 12 L10 AND VACCIN?

=> d bib ab 1-12

L11 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:488873 BIOSIS
DN PREV200200488873
TI VSG-**GPI** anchors of African trypanosomes: Their role in
macrophage activation and induction of infection-associated
immunopathology.
AU Magez, Stefan (1); Stijlemans, Benoit; Baral, Toya; De Baetselier, Patrick
CS (1) Laboratory of Cellular Immunology, Free University of Brussels/Flemish
Interuniversity, Institute for Biotechnology, Paardenstraat 65, 1640, Sint
Genesius Rode: stemagez@vub.ac.be Belgium
SO Microbes and Infection, (July, 2002) Vol. 4, No. 9, pp. 999-1006. print.
ISSN: 1286-4579.
DT Article
LA English
AB African trypanosomes express a glycosylphosphatidyl inositol (**GPI**
)-anchored variant-specific surface glycoprotein (VSG) as a protective
coat. During infection, large amounts of VSG molecules are released into
the circulation. Their interaction with various cells of the immune system
underlies the severe infection-associated pathology. Recent results have
shown that anti-**GPI vaccination** can prevent the
occurrence of this pathology.

L11 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:256288 BIOSIS
DN PREV200000256288
TI Role of **parasite** surface glycoconjugates on induction/regulation
of immune responses and inflammation, elicited during **Trypanosoma**
cruzi infection: Potential implications on pathophysiology of Chagas'
disease.
AU Gazzinelli, Ricardo T. (1); Rodrigues, Mauricio M.; Almeida, Igor C.;
Travassos, Luiz R.
CS (1) Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, MG,
30190-002 Brazil
SO Ciencia e Cultura (Sao Paulo), (Sept. Dec., 1999) Vol. 51, No. 5-6, pp.
411-428. print..
ISSN: 0009-6725.
DT Article
LA English

individual proteins and cannot be attributed to the lack of a **GPI** -anchor. The different intracellular location and post-translational modification of recombinant proteins expressed in insect cells, as compared to the native proteins expressed in **parasites**, and the possible implications for **vaccine** development are discussed.

L11 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:122678 BIOSIS
DN PREV200000122678
TI An early step of glycosylphosphatidyl-inositol anchor biosynthesis is abolished in lepidopteran insect cells following baculovirus infection.
AU Azzouz, Nahid; Kedees, Mamdouh H.; Gerold, Peter; Becker, Stephan; Dubremetz, Jean-Francois; Klenk, Hans-Dieter; Eckert, Volker; Schwarz, Ralph T. (1)
CS (1) Med. Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg Germany
SO Glycobiology, (Feb., 2000) Vol. 10, No. 2, pp. 177-183.
ISSN: 0959-6658.
DT Article
LA English
SL English
AB The expression of recombinant proteins in their native state has become a prerequisite for a variety of functional and structural studies, as well as **vaccine** development. Many biochemical properties and functions of proteins are dependent on or reside in posttranslational modifications, such as glycosylation. The baculovirus system has increasingly become the system of choice due to it capabilities of performing posttranslational modifications and usually high yields of recombinant proteins. The **Toxoplasma** gondii surface antigen SAG1 was used as a model for a glycosylphosphatidyl-inositol (**GPI**)-anchored protein and expressed in insect cells using the baculovirus system. We show that the T. gondii SAG1 surface antigen expressed in this system was not modified by a **GPI**-anchor. In vitro and in vivo studies demonstrate that uninfected insect cells are able to produce **GPI**-precursors and to transfer a mature **GPI**-anchor to nascent proteins. These cells however are not capable to produce **GPI**-precursors following infection. We also show that the biosynthesis of the early **GPI** intermediate GlcNH2-PI is blocked in baculovirus-infected H5 cells, thus preventing the subsequent mannosylation steps for the synthesis of the conserved **GPI** -core-glycan. We therefore conclude that the baculovirus system is not appropriate for the expression of **GPI**-anchored proteins.

L11 ANSWER 5 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:391590 BIOSIS
DN PREV199699113946
TI Aberrant processing and localisation of a **GPI**-anchored malaria surface antigen expressed in the baculovirus system.
AU Eckert, V. (1); Kedees, M. H.; Gerold, P.; Muehlberger, E.; Becker, S.; Holder, A. A.; Klenk, H.-D. (1); Schwarz, R. T. (1)
CS (1) Med. Zentrum Hygiene Med. Mikrobiol., Philipps-Univ. Marburg, Robert-Koch-Str. 17, D-35037 Marburg Germany
SO European Journal of Cell Biology, (1996) Vol. 69, No. SUPPL. 42, pp. 148.
Meeting Info.: 21st Annual Meeting of the German Society for Cell Biology Hamburg, Germany March 24-28, 1996
ISSN: 0171-9335.
DT Conference
LA English

L11 ANSWER 6 OF 12 CABA COPYRIGHT 2003 CABI
AN 95:5863 CABA
DN 940806413

TI XX Annual Meeting on Basic Research in Chagas' Disease (Caxambu, MG, November 9-11, 1993)
CS Memorias do Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brazil.
SO Memorias do Instituto Oswaldo Cruz, (1993) Vol. 88, No. Suppl., pp. 1-271, 316-322.
ISSN: 0074-0276
DT Conference Article; Journal
LA English
AB This annual meeting included 7 mini conferences on: myristoylation of **GPI** anchors in **Trypanosoma** brucei; structural and functional aspects of human immune responses against the major cysteine proteinase from T. cruzi; evolution and mechanism of RNA editing; development and interactions of T. cruzi within the insect vector - some facts and ideas; the calmodulin-ubiquitin associated genes of T. cruzi are required for **parasite** viability; gamma delta -CD3 and CD5 B lymphocytes play a consistent role in the mechanisms leading to Chagas' disease pathology; specific immune regulation of CD4+ T cell-mediated diseases of the central nervous system - insights into the pathogenesis and treatment of experimental autoimmune encephalomyelitis and Theiler's virus-induced demyelinating disease. The publication also includes an interface conference on the structure and replication of kinetoplast DNA, with 11 round table discussions on the following: glycoconjugates of T. cruzi and Leishmania; cellular biology of Leishmania; transialidase; immunological basis of **vaccination** in Leishmania; cells and mechanisms involved in the immunopathology in Chagas' disease; perspectives for elimination of vectorial and transfusional transmission of Chagas' disease in the Americas; pathogenesis of Chagas' disease - basic aspects; molecular genetics of T. cruzi and Leishmania; diagnosis and taxonomy of T. cruzi and Leishmania at the molecular level; chemotherapy of Chagas' disease and leishmaniasis; human Chagas' disease - an overview of recent advances. There are then 63 abstracts on biology and ultrastructure, 97 on biochemistry and molecular biology, 115 on immunology and immunopathology, 25 on chemotherapy and 44 on vectors.

L11 ANSWER 7 OF 12 CABA COPYRIGHT 2003 CABI
AN 90:14520 CABA
DN 900861456
TI Identification and characterization of glycosylphosphatidylinositol-linked Schistosoma mansoni adult worm immunogens
AU Sauma, S. Y.; Strand, M.
CS M. Strand, Johns Hopkins University School of Medicine, Department of Pharmacology and Molecular Sciences, Baltimore, MD 21205, USA.
SO Molecular and Biochemical Parasitology, (1990) Vol. 38, No. 2, pp. 199-209. 40 ref.
ISSN: 0166-6851
DT Journal
LA English
AB Metabolic radiolabelling of adult S. mansoni with [3H]myristic acid showed that the fatty acid is incorporated into more than 15 proteins. Two of these proteins, a 200 000 MW glycoprotein known to be exposed on the surface of the adult worm following praziquantel treatment and a 22 000 MW glycoprotein that showed an enhanced immune reactivity with sera of **vaccinated** mice, were anchored to the adult worm membrane via a glycosylphosphatidylinositol (**GPI**) linkage. Both antigens partitioned preferentially into the detergent phase of Triton X-114 and were susceptible, following immunoaffinity purification, to hydrolysis by phosphatidylinositol-specific phospholipase C (PIPLC) from Bacillus thuringiensis and phospholipase C from Bacillus cereus. Diacylglycerol (DAG) was released following hydrolysis by bacterial PIPLC; however, **Trypanosoma** brucei GPIPLC failed to release the diacylglycerol from either protein. Treatment with nitrous acid generated phosphatidylinositol (PI) from both proteins, and phospholipase D from rat

Suzanna; McConville, Malcolm; Schofield, Louis
CS (1) Walter Eliza Hall Inst. Med. Res., VIC 3050 Australia
SO Proceedings of the National Academy of Sciences of the United States of America, (1997) Vol. 94, No. 8, pp. 4022-4027.
ISSN: 0027-8424.
DT Article
LA English
AB The perturbation of various glycosylphosphatidylinositol (**GPI**)-anchored surface proteins imparts profound regulatory signals to macrophages, lymphocytes and other cell types. The specific contribution of the **GPI** moieties to these events however is unclear. This study demonstrates that purified GPIs of **Plasmodium** falciparum, **Trypanosoma** brucei, and Leishmania mexicana origin are sufficient to initiate signal transduction when added alone to host cells as chemically defined agonists. GPIs (10 nM-1 mu-M) induce rapid activation of the protein tyrosine kinase (PTK) p59-hck in macrophages. The minimal structural requirement for PTK activation is the evolutionarily conserved core glycan sequence Man-alpha-1-2Man-alpha-1-6Man-alpha-1-4GlcN1-6myo-inositol. **GPI**-associated diacylglycerols independently activate the calcium-independent epsilon isoform of protein kinase C. Both signals collaborate in regulating the downstream NF-kappa-B/rel-dependent gene expression of interleukin 1-alpha, tumor necrosis factor (TNF) alpha, and inducible NO synthase. The alkylacyl-glycerol-containing iM4 GIPL of L. mexicana, however, is unable to activate protein kinase C and inhibits TNF expression in response to other agonists, establishing signaling specificity among structurally distinct GPIs. **GPI** alone appears sufficient to mimic the activities of **malaria parasite** extracts in the signaling pathway leading to TNF expression. A mAb to **GPI** blocks TNF induction by **parasite** extracts indicating that **GPI** is a necessary agent in this response. As protozoal GPIs are closely related to their mammalian counterparts, the data indicate that GPIs do indeed constitute a novel outside-in signaling system, acting as both agonists and second messenger substrates, and imparting at least two separate signals through the structurally distinct glycan and fatty acid domains. These activities may underlie aspects of pathology and immune regulation in protozoal infections.

L13 ANSWER 8 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:391590 BIOSIS
DN PREV199699113946
TI Aberrant processing and localisation of a **GPI**-anchored **malaria** surface antigen expressed in the baculovirus system.
AU Eckert, V. (1); Kedees, M. H.; Gerold, P.; Muehlberger, E.; Becker, S.; Holder, A. A.; Klenk, H.-D. (1); Schwarz, R. T. (1)
CS (1) Med. Zentrum Hygiene Med. Mikrobiol., Philipps-Univ. Marburg, Robert-Koch-Str. 17, D-35037 Marburg Germany
SO European Journal of Cell Biology, (1996) Vol. 69, No. SUPPL. 42, pp. 148.
Meeting Info.: 21st Annual Meeting of the German Society for Cell Biology Hamburg, Germany March 24-28, 1996
ISSN: 0171-9335.
DT Conference
LA English

L13 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2003 ACS
AN 1995:319826 CAPLUS
DN 122:98808
TI Cloning and expression of human .beta.2-microglobulin cDNA and the construction of fusion proteins between antigenic epitopes and .beta.2-microglobulin
IN Edwards, Richard Mark; Hunter, Michael George
PA British Bio-Technology Ltd., UK
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2

into phospholipids and other proteins. Myristate analogs are useful for studying the mechanism of **GPI** myristoylation, and they are candidates for antitrypanosomal chemotherapy.

L13 ANSWER 11 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1995:79000 BIOSIS
DN PREV199598093300
TI Glycosylphosphatidylinositol toxin of **Trypanosoma** brucei regulates IL-1-alpha and TNF-alpha expression in macrophages by protein tyrosine kinase mediated signal transduction.
AU Tachado, Souvenir D. (1); Schofield, Louis
CS (1) Walter and Eliza Hall Inst. Med. Res., Post Office, Royal Melbourne Hosp., Parkville 3050, Victoria Australia
SO Biochemical and Biophysical Research Communications, (1994) Vol. 205, No. 2, pp. 984-991.
ISSN: 0006-291X.
DT Article
LA English
AB A purified, structurally defined glycosylphosphatidylinositol (**GPI**) derived from the Variant Surface Glycoprotein (VSG) of **Trypanosoma** brucei, and its biosynthetic precursor P2, was able at submicromolar concentrations to regulate cytokine expression when added directly as pharmacological agonist to host macrophages, by activation of an endogenous protein tyrosine-kinase (PTK) mediated signal transduction pathway. **GPI** induces rapid onset tyrosine phosphorylation of multiple intracellular substrates, within minutes of addition to LPS-nonresponsive cells, followed shortly thereafter by IL-1-alpha secretion. The PTK antagonists genistein and tyrphostin inhibit both tyrosylphosphorylation and cytokine expression. A monoclonal antibody to **GPI** also blocks IL-1-alpha induction by total **parasite** extracts. Thus, as in **malaria** infection, **GPI** may induce the cytokine excess causing certain pathological states associated with trypanosomiasis.

L13 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1994:253515 BIOSIS
DN PREV199497266515
TI Requirements for glycosylphosphatidylinositol attachment are similar but not identical in mammalian cells and **parasitic** protozoa.
AU Moran, Paul; Caras, Ingrid W. (1)
CS (1) Dep. Neurobiol., Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080 USA
SO Journal of Cell Biology, (1994) Vol. 125, No. 2, pp. 333-343.
ISSN: 0021-9525.
DT Article
LA English
AB The general features of the glycosylphosphatidylinositol (**GPI**) signal have been conserved in evolution. To test whether the requirements for **GPI** attachment are indeed the same in mammalian cells and **parasitic** protozoa, we expressed the prototype **GPI**-linked protein of **Trypanosoma** brucei, the variant surface glycoprotein (VSG), in COS cells. Although large amounts of VSG were produced, only a small fraction became **GPI** linked. This impaired processing is not caused by the VSG ectodomain, since replacement of the VSG **GPI** signal with that of decay accelerating factor (DAF) produced **GPI**-linked VSG. Furthermore, whereas fusion of the DAF **GPI** signal to the COOH terminus of human growth hormone (hGH) produces **GPI**-linked hGH, an analogous hGH fusion using the VSG **GPI** signal does not, indicating that the VSG **GPI** signal functions poorly in mammalian cells. By constructing chimeric VSG-DAF **GPI** signals and fusing them to the COOH terminus of hGH, we show

DT Article
LA English
SL English
AB The expression of recombinant proteins in their native state has become a prerequisite for a variety of functional and structural studies, as well as **vaccine** development. Many biochemical properties and functions of proteins are dependent on or reside in posttranslational modifications, such as glycosylation. The baculovirus system has increasingly become the system of choice due to it capabilities of performing posttranslational modifications and usually high yields of recombinant proteins. The **Toxoplasma** gondii surface antigen SAG1 was used as a model for a glycosylphosphatidyl-inositol (**GPI**)-anchored protein and expressed in insect cells using the baculovirus system. We show that the T. gondii SAG1 surface antigen expressed in this system was not modified by a **GPI**-anchor. In vitro and in vivo studies demonstrate that uninfected insect cells are able to produce **GPI**-precursors and to transfer a mature **GPI**-anchor to nascent proteins. These cells however are not capable to produce **GPI**-precursors following infection. We also show that the biosynthesis of the early **GPI** intermediate GlcNH2-PI is blocked in baculovirus-infected H5 cells, thus preventing the subsequent mannosylation steps for the synthesis of the conserved **GPI**-core-**glycan**. We therefore conclude that the baculovirus system is not appropriate for the expression of **GPI**-anchored proteins.

L15 ANSWER 2 OF 3 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 2000059865 EMBASE
TI Processing and localisation of a **GPI**-anchored **Plasmodium** falciparum surface protein expressed by the baculovirus system.
AU Kedees M.H.; Gerold P.; Azzouz N.; Blaschke T.; Shams-Eldin H.; Muhlberger E.; Holder A.A.; Klenk H.-D.; Schwarz R.T.; Eckert V.
CS Prof. R.T. Schwarz, Zent. Hygiene Medizin. Mikrobiol., Philipps-Universitat Marburg, Robert-Koch-Strasse 17, D-35037 Marburg, Germany. schwarz@mailer.uni-marburg.de
SO European Journal of Cell Biology, (2000) 79/1 (52-61).
Refs: 69
ISSN: 0171-9335 CODEN: EJCBDN
CY Germany
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB We describe the expression, in insect cells using the baculovirus system, of two protein fragments derived from the C-terminus of merozoite surface protein 1 (MSP-1) of the human malaria **parasite** **Plasmodium** falciparum, and their glycosylation and intracellular location. The transport and intracellular localisation of the intact C-terminal MSP-1 fragment, modified by addition of a signal sequence for secretion, was compared with that of a similar control protein in which translation of the **GPI**-cleavage/attachment site was abolished by insertion of a stop codon into the DNA sequence. Both proteins could only be detected intracellularly, most likely in the endoplasmic reticulum. This lack of transport to the cell surface or beyond, was confirmed for both proteins by immunofluorescence with a specific antibody and characterisation of their N-glycans. The N-glycans had not been processed by enzymes localised in post-endoplasmic reticulum compartments. In contrast to MSP-1, the surface antigen SAG-1 of **Toxoplasma** gondii was efficiently transported out of the endoplasmic reticulum of insect cells and was located, at least in part, on the cell surface. No **GPI**-anchor could be detected for either of the MSP-1 constructs or SAG-1, showing that the difference in transport is a property of the individual proteins and cannot be attributed to the lack of a **GPI**-anchor. The different intracellular location and post-translational

LA English
SL English
AB A strong activation of macrophages is observed during acute infection with **Trypanosoma** cruzi. Little is known, however, about the **parasite** molecules that are responsible for this early activation of innate immunity. Recent studies have shown the stimulatory activity of protozoan-derived glycosylphosphatidylinositol (**GPI**) anchors on cultured macrophages. In this review, we provide a detailed analysis of the correlation between structure and proinflammatory activity by T. cruzi-derived **GPI** anchors. We also cover the studies that have identified the Toll-like receptor 2 as a functional **GPI** receptor and have partially characterized signaling pathways triggered by T. cruzi-derived **GPI** anchors, which lead to the synthesis of proinflammatory cytokines in macrophages. Finally, we discuss the implications of these findings in resistance and pathogenesis during the infection with T. cruzi.

L16 ANSWER 8 OF 66 MEDLINE DUPLICATE 4
AN 2001364652 MEDLINE
DN 21311900 PubMed ID: 11418678
TI Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan **parasite**.
AU Campos M A; Almeida I C; Takeuchi O; Akira S; Valente E P; Procopio D O; Travassos L R; Smith J A; Golenbock D T; Gazzinelli R T
CS Department of Biochemistry and Immunology, Biological Sciences Institute, Federal University of Minas Gerais and Centro de Pesquisas Rene Rachou, Oswaldo Cruz Foundation, Belo Horizonte, Brazil.
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SO JOURNAL OF IMMUNOLOGY, (2001 Jul 1) 167 (1) 416-23.
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AB Glycosylphosphatidylinositol (**GPI**) anchors and glycoinositolphospholipids (GIPLs) from **parasitic** protozoa have been shown to exert a wide variety of effects on cells of the host innate immune system. However, the receptor(s) that are triggered by these protozoan glycolipids has not been identified. Here we present evidence that **Trypanosoma** cruzi-derived **GPI** anchors and GIPLs trigger CD25 expression on Chinese hamster ovary-K1 cells transfected with CD14 and Toll-like receptor-2 (TLR-2), but not wild-type (TLR-2-deficient) Chinese hamster ovary cells. The protozoan-derived **GPI** anchors and GIPLs containing alkylacylglycerol and saturated fatty acid chains or ceramide were found to be active in a concentration range of 100 nM to 1 microM. More importantly, the **GPI** anchors purified from T. cruzi trypomastigotes, which contain a longer **glycan** core and unsaturated fatty acids in the sn-2 position of the alkylacylglycerolipid component, triggered TLR-2 at subnanomolar concentrations. We performed experiments with macrophages from TLR-2 knockout and TLR-4 knockout mice, and found that TLR-2 expression appears to be essential for induction of IL-12, TNF-alpha, and NO by **GPI** anchors derived from T. cruzi trypomastigotes. Thus, highly purified **GPI** anchors from T. cruzi **parasites** are potent activators of TLR-2 from both mouse and human origin. The activation of TLR-2 may initiate host innate defense mechanisms and inflammatory response during protozoan infection, and may provide new strategies for immune intervention during protozoan

of xenografts transplanted from nonprimate donors to humans, specific inhibitors of .alpha.-galactosyltransferases are of broad interest. Using **Trypanosoma** brucei, a protozoan **parasite** causing sleeping sickness and Nagana, we have a very useful model system for the investigation of .alpha.-galactosyltransferase inhibitors, since the variant surface glycoprotein (VSG) accounts for about 10% of the total cell protein and this **parasite** expresses many different galactosyltransferases including the one catalyzing the formation of the Gal.alpha.1.fwdarw.3Gal epitope. In order to study inhibition of galactosylation on the VSG from **Trypanosoma** brucei, we designed, synthesized and tested substrate analogs of trypanosomal .alpha.-galactosyltransferases. Effective inhibitors were a pair of diastereoisomeric UDP-galactose analogs, in which the galactose residue is linked to UDP via a methylene bridge rather than an ester linkage. Hence, galactose cannot be transferred to the resp. acceptor substrate VSG or the synthetic acceptor substrate Man.alpha.1.fwdarw.6Man.alpha.1S-(CH2)7-CH3, which was previously proven to replace VSG effectively. Inhibitors have been prepd. starting from 1-formyl galactal. The final condensation was performed using UMP morpholidate leading to a pair of diastereomeric compds. in 39% or 30% yield, resp. These compds. were tested using .alpha.-galactosyltransferases prepd. from T. brucei membranes and lactose synthetase from bovine milk. While the KM-value for UDP-galactose was detd. as 59 .mu.M on bovine lactose synthetase, the KI-values for both inhibitors were 0.3 mM and 1.1 mM resp., showing that these inhibitors are unable to inhibit enzyme activity significantly. However, using the N-**glycan** specific .alpha.-galactosyltransferase from trypanosomes, the KM-value was detd. as 20 .mu.M, while the KI-value were 34 .mu.M and 21 .mu.M resp. Interestingly, other trypanosomal .alpha.-galactosyltransferases, which modify the **GPI** membrane anchor, are 2 orders of magnitude less effected by the inhibitor.

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L16 ANSWER 17 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:60544 BIOSIS
DN PREV200100060544
TI DRMs, secretion and **lipid** architecture in trypanosomatidae.
AU Denny, P. W. (1); Field, M. C. (1); Smith, D. F. (1)
CS (1) Wellcome Trust Laboratories for Molecular Parasitology, Dept. Biochemistry, Imperial College, London UK
SO Biochemical Society Transactions, (October, 2000) Vol. 28, No. 5, pp. A477. print.
Meeting Info.: 18th International Congress of Biochemistry and Molecular Biology Birmingham, UK July 16-20, 2000
ISSN: 0300-5127.
DT Conference
LA English
SL English

L16 ANSWER 18 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:122678 BIOSIS
DN PREV200000122678
TI An early step of glycosylphosphatidyl-inositol anchor biosynthesis is abolished in lepidopteran insect cells following baculovirus infection.
AU Azzouz, Nahid; Kedees, Mamdouh H.; Gerold, Peter; Becker, Stephan; Dubremetz, Jean-Francois; Klenk, Hans-Dieter; Eckert, Volker; Schwarz, Ralph T. (1)
CS (1) Med. Zentrum fuer Hygiene und Medizinische Mikrobiologie, Philipps-Universitaet Marburg, Robert-Koch-Strasse 17, D-35037, Marburg Germany
SO Glycobiology, (Feb., 2000) Vol. 10, No. 2, pp. 177-183.
ISSN: 0959-6658.
DT Article

were 34 muM and 21 muM respectively. Interestingly, other trypanosomal alpha-galactosyltransferases, which modify the **GPI** membrane anchor, are 2 orders of magnitude less effected by the inhibitor.

L16 ANSWER 28 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 1999187938 EMBASE
TI Biosynthesis of glycosylphosphatidylinositols in mammals and unicellular microbes.
AU Tiede A.; Bastisch I.; Schubert J.; Orlean P.; Schmidt R.E.
CS R.E. Schmidt, Department of Clinical Immunology, Hannover Medical School, D-30625 Hannover, Germany
SO Biological Chemistry, (1999) 380/5 (503-523).
Refs: 169
ISSN: 1431-6730 CODEN: BICHF3
CY Germany
DT Journal; General Review
FS 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry
LA English
SL English
AB Membrane anchoring of cell surface proteins via glycosylphosphatidylinositol (**GPI**) occurs in all eukaryotic organisms. In addition, **GPI**-related glycophospholipids are important constituents of the **glycan** coat of certain protozoa. Defects in **GPI** biosynthesis can retard, if not abolish growth of these organisms. In humans, a defect in **GPI** biosynthesis can cause paroxysmal nocturnal hemoglobinuria (PNH), a severe acquired bone marrow disorder. Here, we review advances in the characterization of **GPI** biosynthesis in **parasitic** protozoa, yeast and mammalian cells. The **GPI** core structure as well as the major steps in its biosynthesis are conserved throughout evolution. However, there are significant biosynthetic differences between mammals and microbes. First indications are that these differences could be exploited as targets in the design of novel pharmacotherapeutics that selectively inhibit **GPI** biosynthesis in unicellular microbes.

L16 ANSWER 29 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:203614 BIOSIS
DN PREV200000203614
TI Ether **lipids** X ceramides in **GPI** anchors of trypanosomatids: First round.
AU Heise, N. (1); Lima, A. C. P. (1); Hart, D. T.; Mendonca-Previato, L. (1); Previato, J. O. (1)
CS (1) Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, 21944-970, Rio de Janeiro, RJ Brazil.
SO Memorias do Instituto Oswaldo Cruz, (Nov., 1999) Vol. 94, No. SUPPL. 2, pp. 39.
Meeting Info.: XXVI Annual Meeting on Basic Research in Chagas' Disease and the XV Annual Meeting of Brazilian Society of Protozoology. Caxambu, Brazil November 09-11, 1999
ISSN: 0074-0276.
DT Conference
LA English
SL English

L16 ANSWER 30 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:210885 BIOSIS
DN PREV200000210885
TI Diversity in the **lipid** of the **GPI**-anchors in **Trypanosoma** cruzi.
AU Lederkremer, R. M. (1)
CS (1) CIHIDECAR, Departamento de Quimica Organica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires Argentina

acidic repetitive proteins of **Trypanosoma** brucei.
AU Mehlert, Angela; Zitzmann, Nicole; Richardson, Julia M.; Treumann, Achim; Ferguson, Michael A. J. (1)
CS (1) Dep. Biochem., Univ. Dundee, Dundee DD1 4HN UK
SO Molecular and Biochemical Parasitology, (March 1, 1998) Vol. 91, No. 1, pp. 145-152.
ISSN: 0166-6851.
DT Article
LA English
AB **Trypanosoma** brucei, in common with the other African trypanosomes, exhibits unusual cell-surface molecular architecture. The bloodstream form of the **parasite** is coated with a continuous layer of approximately five million variant surface glycoprotein (VSG) dimers that provide the **parasite** with a macromolecular diffusion barrier to guard against lysis by the alternative complement pathway. The procyclic form of the **parasite** has a more diffuse cell-surface coat made up of approximately 2.5 million copies of procyclic acidic repetitive protein (PARP). Within the VSG and PARP coats exist lower-abundance surface glycoproteins such as receptors and nutrient transporters. Both the VSG molecules and the PARP molecules are attached to the membrane via glycosylphosphatidylinositol (**GPI**) membrane anchors and the VSGs and one form of PARP are N-glycosylated. In this article, the structures of the N-glycans and the **GPI** anchors of T. brucei VSGs and PARPs are reviewed and simple models of the surfaces of bloodstream and procyclic trypomastigotes are presented.

L16 ANSWER 33 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 97144891 EMBASE
DN 1997144891
TI Proteins with glycosylphosphatidylinositol (**GPI**) signal sequences have divergent fates during a **GPI** deficiency. GPIs are essential for nuclear division in **Trypanosoma** cruzi.
AU Garg N.; Tarleton R.L.; Mensa-Wilmot K.
CS K. Mensa-Wilmot, Department of Cellular Biology, Biological Sciences Bldg., University of Georgia, Athens, GA 30602, United States. mensawil@cellmate.cb.uga.edu
SO Journal of Biological Chemistry, (1997) 272/19 (12482-12491).
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ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB Glycosylphosphatidylinositols (GPIs) are membrane anchors for cell surface proteins of several major protozoan **parasites** of humans, including **Trypanosoma** cruzi, the causative agent of Chagas' disease. To investigate the general role of GPIs in T. cruzi, we generated **GPI**-deficient **parasites** by heterologous expression of T. brucei **GPI**-phospholipase C. Putative protein- **GPI** inter mediates were depleted, causing the biochemical equivalent of a dominant-negative loss of function mutation in the **GPI** pathway. Cell surface expression of major **GPI**-anchored proteins was diminished in **GPI**-deficient T. cruzi. Four proteins that are normally **GPI**-anchored in T. cruzi exhibited different fates during the **GPI** shortage; Ssp-4 and p75 were secreted prematurely, while protease gp50/55 and p60 were degraded intracellularly. These observations demonstrate that secretion and intracellular degradation of **GPI**-anchored proteins may occur in the same genetic background during a **GPI** deficiency. We postulate that the interaction between a protein-**GPI** transamidase and the COOH-terminal **GPI** signal sequence plays a pivotal role in determining the fate of these proteins. At a nonpermissive **GPI**